Report of the Haffkine Institute

For the Year 1936

BY

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Director, Haffkine Institute

BOMBAY

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CONTENTS

PART I.

	Page.
Staff	1—2
General Review	3—9
Social Life at the Institute	10

SECTION I.

Vaccine and Serum Department	11—14
A. Haffkine's Plague Vaccine	11—12
(1) Manufacture	11
(2) Distribution	12
B. Cholera Vaccine	13
C. Other Vaccines	13—14
D. Sera	14

SECTION II.

Epidemiology of Plague	15—20
A. Training of Health Personnel	15
B. Technical Assistance	15
C. Examination of Bombay City Rats	15—17
D. Examination of Rats from Steamers in the Bombay Harbour ...	17
E. Preparation and Supply of Exhibits on Plague Preventive Measures ...	18
F. The Manufacture of Approved Rat Traps	18
G. Isolation of an Organism from Rats showing Lesions like those of Plague.	18—20

SECTION III.

Department of Bio-Chemistry	20—22
(1) Nutritional Work	20
(2) Concentration of Anti-plague Serum	20
(3) Normal Clinical Standards for Indians	20
(4) Shrinkage of Red Blood Cells due to Potassium Oxalate	21
(5) Estimation of Ascorbic Acid Content in the Blood and Urine of normal Indians	21
(6) Relation between Coagulation time and the Concentration of Russell's Viper Venom	21
(7) Chemical Analyses	21—22

SECTION IV.

Department of Pharmacology	22—24

SECTION V.

Department of Public Health Chemistry and Bacteriology	24
A. Chemical Work	24
B. Bacteriological Work	24
C. Miscellaneous Work	24

Page.

SECTION VI.

Diagnostic Department ... ... 25—28
A. Bacteriological Section ... ... 25—27
B. Pathological Section ... ... 27—28

SECTION VII.

Anti-rabic Department ... ... 28—30

SECTION VIII.

Income and Expenditure 30

PART II.

Research Work—
I. Plague Vaccine ... ... 31—39
A. The Relative Protective Power of Broth Vaccines incubated at 27°C. and those incubated at 37°C. ... ... 31—32
B. The Relative Protective Power of Agar Vaccines incubated at 27°C. and those incubated at 37°C. ... ... 32—33
C. Live Avirulent Broth Vaccines compared with Heat-killed Broth Vaccines ... ... 33—34
D. Live Avirulent Agar Vaccines compared with Heat-killed Agar Vaccines 34
E. Effect of the Method of Killing of Cultures on the Potency of the Resultant Vaccine ... ... 35—37
F. Effect of Storage on the Potency of Live Avirulent Vaccines ... 37—38
G. Growth of Past. pestis at different Temperatures of Incubation and its Possible Relationship to the Start and End of Epidemics during Certain Seasons 38
H. Immunisation of Different Laboratory Animals with Heat-killed Plague Vaccines ... ... 38—39
II. Anti-plague Serum ... ... 39—40
III. Serology of Plague ... ... 41—43
A. Agglutination Reaction... ... ... 41—42
B. Precipitin Reaction ... ... 42—43
C. Hæmolysin Production by Past. pestis 43
IV. Epidemiology of Plague ... ... 43—45
(1) Susceptibility to Plague of Rats from Different Parts of India ... 43—44
(2) Transmission by Heredity of Acquired Immunity 44
(3) Immunity to Plague of Bombay Rats 45
V. Nutrition, Preliminary Experiments ... ... 45—46
VI. Ascorbic Acid Content in the Blood of normal Indians 46
VII. Ascorbic Acid Content in the Urine of normal Indians 46
VIII. Extent of Shrinkage of the Red Cells in Blood due to the Use of Potassium Oxalate ... ... 46—47
IX. Investigations on the Bio-Chemistry of Plant Glycolysis ... ... 47—48
X. Carbohydrate Reactions of Meningococcus ... ... 48—49
XI. Pharmacology of Plasmoquine 49

Page.

XII. Studies in Acetylcholine ... ... 50—51

A. Action of Acetylcholine on the Brain 50

B. Relation between Acetylcholine Content of the Brain and the Choline Esterase of the Serum 50

C. Choline-esterase ... ... 50—51

XIII. Pharmacological Action of Flavones with special reference to their Anthelminthic Effect 51

XIV. Prophylaxis of Chicken-pox by Inoculation with Vesicular Fluid ... 52

XV. Investigations into the Mosquitocidal Value of Indigenous Drugs ... 52—54

XVI. The Prevention of Hæmoglobinuria in P. knowlesi Infections by Methylene Blue and its Curative Value when combined with Quinine Salts 54

List of Papers published from the Haffkine Institute during the year 1936 ... 54—55

PART III.

Statistics of Anti-rabic Inoculation for 1935 ... ... 57—92

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1936.

PART I.

STAFF.

Director.

Lt.-Col. S. S. Sokhey, M.A., M.D. (Edin.), D.T.M. & H. (Eng.), F.I.A.S., F.N.I., I.M.S.

Assistant Directors.

Major R. C. Wats, M.D., Ch.B. (L'pool), D.P.H., D.T.M., I.M.S., from 1st January to 27th July and from 13th November to 31st December.

Dr. P. M. Wagle, M.B. (Bom.), D.Bac. (Manch.), D.P.H. (Lond.), D.T.M. (L'pool).

Captain J. R. Dogra, M.D., Ch.B. (Edin.), D.T.M., I.M.S., attached to Haffkine Institute, was Assistant Director from 28th July to 12th November and Supernumerary Officer from 13th November to 31st December.

Department of Pharmacology.

Dr. B. B. Dikshit, Ph.D. (Edin.), M.R.C.P. (Edin.), D.P.H.

Department of Bio-chemistry.

Mr. M. A. Malandkar, M.A., M.Sc.

Mr. S. K. Gokhale, B.A., M.Sc., A.I.I.Sc.

Anti-rabic Department.

Military Assistant Surgeon Hugh Maurice, I.S.O., I.M.D.

Mr. R. K. Gokhale, L.C.P.S., S.M.S.

Anti-plague Inquiry.

Mr. R. G. Sathe, G.B.V.C., Veterinary Assistant Surgeon.

Voluntary Research Workers.

Dr. R. Row, O.B.E., M.D., D.Sc. (Lond.).

Dr. S. N. Gore, L.M. & S. (Bom.).

Dr. H. S. Mahal, Ph.D., M.Sc.

Mr. M. Sadashiv Rao, M.A.

Medical and Scientific Assistants.

Rao Bahadur G. D. Chitre, L.M. & S., B.M.S. (I).
Dr. N. P. Dalal, M.B., B.S. (Bom.), D.P.H., D.T.M. & H. (Eng.), B.M.S. (I).
Mr. J. P. Menezes, M.B., B.S., B.M.S. (II).
Mr. K. H. Bharucha, B.A., B.Sc.
Mr. P. H. Kanade, L.C.P.S., S.M.S., from 1st January to 15th November.

Technical Staff.

Mr. A. Petty ...	... (Vaccine Department).
Mr. T. K. Puduval, B.A.	... (Do. do.).
Mr. A. S. Narasimham, B.A.	... (Do. do.).
Mr. R. Sadashivan, B.Sc.	... (Do. do.).
Mr. M. K. Habbu, B.Sc.	... (Do. do.).
Mr. A. J. Naidu ...	... (Pathology).
Mr. R. D. Panwalkar, A.M.	... (Artist and Photographer).
Mr. M. V. Kamat, B.A.	... (Librarian).

Office Staff.

Mr. C. R. Deshpande	... Head Clerk.
Mr. D. N. Mhaskar	... Accountant.
Mr. D. S. Dixit ...	... Store-keeper.
Mr. M. S. Subramanian, B.A.	... Stenographer.
Mr. S. Y. Nabar ...	... Packing.

Junior Staff, subordinates and menials number 116.

GENERAL REVIEW.

The Institute continued to function during the year as (*a*) the centre for the manufacture of plague vaccine for the whole of India and of other prophylactic vaccines for the Presidency, (*b*) the Provincial Bacteriological Laboratory for the Bombay Presidency, (*c*) the centre for the manufacture of anti-rabic vaccine for Bombay Presidency and adjoining areas and treatment centre for Bombay City, and (*d*) a centre for research. Researches in plague and pharmacology were carried out with the generous assistance of the Indian Research Fund Association.

DEPARTMENT OF VACCINES AND SERA.

The demand for plague vaccine dropped somewhat during the year; 881,054 doses (i.e., 2,643,162 c.c.) were issued as compared with 1,017,044 doses during the previous year. The lowered demand happily is due to lessened incidence of the disease, and not to any falling off in the popularity of the vaccine as a prophylactic measure. There were 23,873 attacks of plague in 1936 against 41,042 attacks in 1935. The demand for the vaccine continues to be high in relation to the incidence of plague.

The outbreak of cholera in the Presidency necessitated heavy demands on the Institute for the supply of cholera vaccine and put a serious strain on the reduced staff. 705,621 doses of the vaccine were issued during the year. In addition, 16,331 doses of meningococcal vaccine and 5,746 doses of typhoid vaccine were issued.

The supply of reliable sera to Government hospitals at moderate prices has been a problem demanding solution for some time. About the middle of the year, the Institute was called upon to undertake this supply. To meet the immediate needs, the Institute has been fortunate enough in obtaining sera at very moderate prices from one of the leading serum institutes—State Serum Institute of Copenhagen. To keep the costs down still further sera are obtained in bulk and bottled at the Institute. The problem can be met finally only by the Institute producing its own sera. The matter is under investigation.

PLAGUE RESEARCHES.

(1) *Plague Vaccine.*—Using his standardised quantitative method for measuring the virulence of the plague bacillus and his method for measuring the protective value of plague vaccines, Sokhey continued the researches on the plague vaccine. The work has yielded some very interesting results. (For details see Part II.) After confirming, by further work, his previous observation that *broth vaccines incubated at 27°C. are more potent than those incubated at 37°C.*, he proceeded to investigate the problem as it affects agar vaccines. He finds that *agar vaccines incubated at 37°C. are more potent than agar vaccines incubated at 27°C:* He points out that the difference to a large extent is only apparent. Certain

observations have led him to believe that the potency of a vaccine depends largely on the number of organisms and their size, i.e., the bulk of organisms, in a vaccine. In the case of broth, the growth of organisms is self-limited, and the growth of the plague bacillus at 37°C. is much less than at 27°C. In the case of agar vaccines the number of organisms are varied at will and in all the experiments reported here the agar vaccines had equal number of organisms. But the size of the organism at 37°C. is larger than at 27°C. He does not think that the presence or absence of capsular material affects the issue, because Sokhey and Maurice have been able to show by staining that the capsule is present even when organisms are incubated at as low a temperature as 5°C.

Comparison of live avirulent vaccines and heat-killed vaccines has also yielded interesting results. *In the case of broth vaccines, heat-killed vaccines incubated at 27°C. have proved to be more potent than live avirulent vaccines incubated at 27°C. or at 37°C. but in the case of agar vaccines, live avirulent vaccines incubated at 37°C. have proved to be much more potent than heat-killed vaccines.* It is, however, to be noted that the order of potency of the best live avirulent agar vaccine was the same as that of the heat-killed broth vaccines incubated at 27°C.

The study of the effect of the method of killing of cultures on the potency of the resultant vaccines has produced results of biological significance besides providing indications of great practical value. Here broth and agar vaccines behave exactly alike and the temperature of incubation is the important factor. In the case of vaccines incubated at 27°C. the best results are obtained when killing is done by the application of heat at 55°C. for 15 minutes. Such a vaccine is about twice as potent as a vaccine killed by the addition of phenol (0·5 per cent.) without the application of heat and about 4 times as good as a vaccine killed by the addition of formalin (0·025 per cent.). Killing by the application of heat at 60°C. for 1 hour reduces the potency of the resultant vaccine to about 1/7th of the potency of the vaccine killed at 55°C. for 15 minutes. *The extraordinary phenomenon is that if the cultures are incubated at 37°C., the method of killing does not affect the potency of the resultant vaccines.* This phenomenon is being studied further for a possible explanation. Indications are that the state of the bacterial protein is responsible for this phenomenon. When cultures are incubated at 27° C., the bacterial protein, in solution or otherwise, is still largely in a coagulable form, but when the cultures are incubated at 37° C., the protein breaks up into a form in which it is no longer easily coagulable. But if this process of breaking up goes further, as in the case of lysis with bacteriophage, it loses its antigenic property entirely, as was shown in the last report.

These are observations of considerable theoretical and practical significance and indicate further experimental work to finally decide the method of obtaining the most potent plague vaccine. It will be noticed that Sokhey has not been able to compare broth vaccines directly with agar vaccines on the basis of the bacterial content of the vaccines, as he has not yet successfully solved the problem of

measuring the bacterial content of a broth vaccine, because during the long period of incubation organisms continue to go into solution. However from the practical point of view such a comparison is perhaps not necessary. The question of the "best" vaccine will be solved by the production of a vaccine having the greatest possible protective power and the minimum of toxicity. This work offers hope of a final solution of the problem.

But it may be noticed that the work reported in Part II of this report already indicates that when vaccines are made by growing the organisms in broth, whether a virulent strain of organisms is used or an avirulent one, whether the growths are incubated at 27°C. or 37°C. and whether the growths are killed by the application of heat (55°C. for 15 minutes), or by the addition of phenol (0·5 per cent.), or by the addition of formalin (0·025 per cent.), the difference between the best and the worst vaccine tends to be low, 1 to 7·5 (0·002 c.c. to 0·015 c.c.). But when the vaccines are made from growths on agar, and the same strains, the same temperatures of incubation and the same methods of killing are employed as in the case of broth vaccines, the difference between the best vaccine and the worst tends to be high, 1 to 100 (·001 c.c. to 1 c.c.), and the best agar vaccine is not much better than the best broth vaccine. The issue will be decided by a measurement of toxicity of the vaccines.

This work raises another issue of interest, that is, the relationship between the serological reactions and the phenomenon of active immunity. Schutze (1932), by serological studies, came to the conclusions that the incubation of plague cultures at 37°C. produced an antigen which was more readily effected by heat (heat labile) than the antigen developed by incubation at 27°C. Measurement of active immunity by the biological method shows that incubation of cultures at 37°C. produces more heat-stable vaccines than incubation at 27°C. In the case of cultures incubated at 27°C., 5°C. rise in the temperature of killing, i.e., 55°C. to 60°C., reduces the potency of the resultant vaccine to 1/7th, while the same increase in the killing temperature does not affect at all the vaccines incubated at 37°C. Preliminary experiments show that a vaccine made from a culture incubated at 37°C. even when heated at 100°C. for 1 hour retains considerable protective power.

The view that "no dead vaccine whatever conferred satisfactory protection on guinea-pigs and wild rats" has also been investigated by Sokhey. He finds that this view also arose from defective experimental work and that heat-killed vaccines do confer a very high degree of immunity on those animals. Using a heat-killed vaccine, he has found that very high protection in the case of guinea-pig, wild rat and white rat can be obtained with doses of 0·01 c.c., 0·025 c.c. and 0·025 c.c. respectively, and that even these doses are not the minimum protective doses, which, it appears, will be still smaller.

(2) *Anti-plague Serum.*—Work has been continued and the use of better methods of immunisation has resulted in the production of a serum twice as potent as the serum produced last year which

gave such encouraging results in the field test at the beginning of the year. Opportunity is awaited to test this new serum in the field.

(3) *Serology of Plague.*—The search for some suitable test-tube method which could take the place of the biological method to test the potency of anti-plague serum produced at the Institute has been continued. Wats has investigated the agglutination reaction, but this reaction does not seem to be sensitive enough for the purpose in view. Wagle has been more successful with the precipitin reaction.

(4) *Epidemiology of Plague.*—Pandemics of plague separated by four centuries or so is a well authenticated phenomenon. These pandemics have ravaged the world, have smouldered here and there for long or short periods and have then come to an end, as they started, of natural causes. The explanation of this phenomenon has always intrigued observers, but no adequate explanation has been forthcoming. The susceptibility of rats to plague was rightly believed to be an important factor, but no exact measurements could be made for lack of the knowledge necessary to prepare a constant standardised infective dose of plague for suitable laboratory experiments. The success at evolving a constant infective dose for the purpose and the present comparatively quiescent period after a severe pandemic in India offered a suitable opportunity of studying the phenomenon.

The results already reported have clearly indicated that the rats in localities where serious epidemics have raged are highly resistant to plague infection. In fact the resistance of the rats seems to be roughly proportional to the intensity of the epidemic of the locality. This resistance can be attributed to several factors. It has been believed that even when plague among human beings has come to an end in a particular locality, the disease continues to smoulder among rats and this continued mild epizootic gives the rats high resistance to plague. But the daily examination of a large number of rats caught in the City of Bombay has shown that this explanation is not correct. For the last 2 years, no rats have been found in the City that showed any signs of infection whatsoever, yet the rat population of Bombay is very highly resistant. The other hypothesis was that rats during an epidemic in a locality acquired immunity and transmitted it to their young. Sokhey has investigated this phenomenon by using white mice. This animal has no natural resistance to plague whatsoever. The young born to parents who had been fully protected against plague showed no immunity whatsoever indicating that acquired immunity is not transmitted.

During their observations, Sokhey and Chitre had obtained rats from Dharamsala, a hill station in the Himalayas, where plague has not been known to have occurred during the present pandemic. Some of the rats obtained from this place showed high resistance to plague, indicating that there exist in nature strains of rats which are naturally immune or resistant to plague. The probable explanation of the coming to an end of an epidemic in

a locality would seem to be that all or most of the susceptible strains of rats die out leaving behind naturally resistant strains. How do fresh pandemics or epidemics start then? During the year these workers have made one observation which throws some light on this problem. During this investigation they had worked entirely with the house rat (*Rattus rattus*) as it had been observed that this species of rat was the one chiefly responsible for the spread of plague to human beings. However, some workers had observed that in some localities the small field rat (*Gunomys varius*) had been responsible for the spread of the disease. Therefore a batch of *Gunomys varius* obtained from the City of Bombay was examined, and they were surprized to find that this species of rats was highly susceptible though the Bombay *Rattus rattus* was highly resistant. This observation is of great significance. If and when this species of rat becomes more numerous or replaces the house rat in human habitations, the stage would be set for fresh epidemics. These workers consider it highly desirable that this question should be studied further. The fact cannot be overstressed that the phenomenon of plague is not yet fully understood and the present time is the most suitable one for further study. It is a comparatively quiescent period after a severe pandemic with just a few cases of plague here and there. These factors make the present period an ideal one for further study and any expenditure that may be incurred would be well worth it. If this period once passes without our acquiring all the knowledge we need to understand the phenomenon fully and thereby devising intelligent means of preventing fresh pandemics. Unless deliberately prevented, pandemics are likely to come again as they have come in the past.

Department of Pharmacology.

During the year the department has been responsible for important research work. New synthetic anti-malarials had been in use for the treatment of malaria for some time. Plasmoquine was the first of these and though a large number of papers describing clinical use of plasmoquine have been published, a complete study of the pharmacology of the drug had not been made. Dikshit investigated the pharmacological action of this drug and described the effects produced on different organs of the body after administration of large doses to experimental animals. His results show that plasmoquine affects most of the systems of the body but more particularly the circulatory and the digestive systems. The two important actions are formation of methæmoglobin which leads to cyanos s and fatty degeneration of the liver. These actions are produced by administration of toxic doses of the drug.

As in the case of quinine, treatment of malaria during pregnancy by plasmoquine has raised a certain amount of controversy. Dikshit undertook to investigate this problem and his preliminary results tend to show that plasmoquine does not produce abortion in experimental animals, even when given in doses which are considerably higher than those used in therapeutics. It appears

from his studies that as far as continuation of pregnancy is concerned, plasmoquine would not in any way interrupt the normal course.

During the last three or four years, the role played by acetylcholine as a physiological agent concerned in chemical transmission at nerve endings has been described by several prominent physiologists. Whether acetylcholine plays the same role in the central nervous system is a doubtful proposition. Dikshit has been trying to get evidence to show that acetylcholine is liberated at the synapsis in the central nervous system. Three years ago he obtained some indirect evidence which suggested this possibility but he has not been able to present any direct evidence regarding liberation of acetylcholine in the central nervous system or in the cerebro-spinal fluid. He is also investigating the problem of the action of acetylcholine by studying an enzyme which destroys acetylcholine. These studies are only in their preliminary stages.

In addition, in view of the intended enactment of the necessary legislation for the control of the sale and manufacture of drugs, in India, the department has been making arrangements for the biological assay of drugs. Standards maintained at the National Institute for Medical Research, Hampstead, have been obtained and from them standards have been established for the Institute. Some samples of Tincture of Digitalis were examined during the year. One sample was only 25 per cent. of the standard strength. A sample of Tincture of Strophanthus examined had a potency of only 1/10th of what it should possess.

The department is still on a temporary basis and is maintained by the Indian Research Fund Association. The value to the Presidency of such a department cannot be overstated and it is hoped Government will find means to put it on a permanent basis.

Department of Bio-Chemistry.

The department continued to be of value both to the workers in the Institute for chemical aid in their work as well as to practitioners and hospitals for the examination of clinical material. In addition, the department has continued to work on establishing the normal clinical standards for Indians and is now engaged on nutritional research work.

Diagnostic Department and Department of Public Health Chemistry and Bacteriology.

As some of the city hospitals are now well-equipped with their own laboratories, these departments are entrusted only with the work that they themselves cannot carry out. Though the work is considerably less than before, these departments are still proving of great value. During the year the department examined over 8,000 specimens sent by hospitals and dispensaries and of these examinations for Wassermann Reaction were 4,000.

Anti-Rabic Department.

Since the Anti-rabic Department was opened in 1922, the popularity of the anti-rabic treatment has grown and in keeping with the policy of making the treatment freely available to the public there are now 69 treatment centres in the Presidency. Five of these were opened during the year. In 1922, 243 treatments were issued to the out-centres and 429 persons were fully treated at this Institute, while during 1936, 5,259 treatments were issued to the out-stations and 2,331 persons were fully treated at the Institute. All this has meant enormously increased work which has now grown beyond the capacity of the present staff.

The yearly increase in the number of persons that receive anti-rabic treatment at this Institute is due partly to the popularity of the anti-rabic treatment and to a great extent to the large number of stray dogs that enter the city from the suburbs and contract rabies. From the admissions it is seen that the largest number of persons bitten are from the thickly populated areas and it is in these areas that stray dogs predominate. From Bombay City, excluding the suburbs, during the year under review 87 dogs' brains were received and examined. 80·4 per cent. of these were found to be rabid. This figure only represents a fraction of the incidence of rabies among dogs in Bombay City as all the dogs dying of the disease are not brought to the Institute. Even this figure discloses a very serious problem and demands immediate measures to prevent the city from being overrun by stray dogs.

Budget.

The working of the Institute during the financial year 1936-37 showed an excess of expenditure over receipts of Rs. 8,253; the receipts were Rs. 3,03,817 and the expenditure Rs. 3,12,070. (See Part I, Section VIII). But the four year period, 1933-1937, including the year under review, shows an average annual credit balance of Rs. 38,894, the average annual receipts being Rs. 3,47,634 and the average annual expenditure Rs. 3,08,740. The working of the Institute for this four year period contrasts very favourably with the previous four year period 1929 to 1933, which showed an average annual excess of expenditure over receipts of Rs. 60,723, allowing for the closing of the Pharmacological Unit and the change in price of plague vaccine. The actual average annual excess of expenditure over receipts for this period was Rs. 1,05,264, the average annual receipts being Rs. 2,47,987 and the average annual expenditure Rs. 3,53,251. This favourable change in the working costs of the Institute was brought about by effecting far reaching economies and by taking up the manufacture of additional biological products, from the commencement of the financial year 1933-34.

SOCIAL LIFE AT THE INSTITUTE.

The Critics Club.—Originally started in 1924, the Club has proved of great value in maintaining collective interest in the scientific and the social activities of the Institute. At the scientific meetings held during the year the following papers were read by members of the staff:—

(1) Measurement of the Immunising Value of Plague Vaccines, by Lt.-Col. Sokhey.

(2) Serology of Plague, Agglutination Reaction, by Major Wats.

(3) Hæmoglobin, Red Cell Count and Cell Volume in Normal Indians, by Mr. Gokhale.

(4) "Capsule or Envelope"?—A dissertation on the so-called Envelope of *Past. pestis,* by Dr. Menezes.

(5) Technique for the Biological Estimation of some Fat Soluble Vitamins, by Mr. Malandkar.

(6) Are Effects of Nerve Stimulation due to liberation of Chemical Substances? by Dr. Dikshit.

(7) Discussion of future work at the Institute by the Staff.

The Critics Tennis Club and the Haffkine Institute Sports Club had very successful seasons. This year Lt.-Col. Sokhey presented a silver cup for the mixed doubles tournament which has added to the popularity of the Tennis Club. Both the Mackie Cup and the Sokhey Cup tournaments were well contested and proved a great success.

A long felt want has been met by the setting apart of a Lunch Room in the Institute. It permits the members of the Staff to have their mid-day meal and tea in a room away from their work rooms. It also serves as a common room.

Haffkine Institute Co-operative Credit Society.—To discourage the employees from incurring indiscriminate debts and paying exorbitant rates of interest to money-lenders, a Co-operative Society was established during the year. Though it is in its infancy, the Society has proved of decided benefit to its members. Apart from encouraging thrifty habits, the Society provided loans at a reasonable rate of interest to 65 persons.

SECTION I.

VACCINE AND SERUM DEPARTMENT.

A. *Haffkine's Plague Vaccine.*

1. *Manufacture.*—Owing to a general decline in the prevalence of plague and the absence of serious epidemic outbreaks during the year under report there was a marked fall in the demand for the plague vaccine. 881,056 doses were issued during the year against 1,017,044 doses in the previous year. But the quinquennial demand for the period 1932-1936 remained undiminished being 6,636,392 doses.

The number of doses of plague vaccine despatched monthly during the years 1935 and 1936 are detailed below :—

	1935.	*1936.*
January	134,050	92,957
February	203,814	77,525
March	120,604	104,134
April	71,738	15,748
May	38,372	775
June	816	48,383
July	3,861	53,897
August	23,794	65,668
September	132,067	92,984
October	93,296	70,926
November	81,110	82,474
December	113,522	175,585
Total ...	1,017,044	881,056

From the opening of the laboratory to the end of 1936 a total of 40,910,371 doses has been issued. The output of the vaccine in quinquennial periods has been as follows :—

Years.	Doses.
1897-1901	2,867,041
1902-1906	3,455,537
1907-1911	3,584,262
1912-1916	4,567,366
1917-1921	6,667,894
1922-1926	7,202,636
1927-1931	5,929,243
1932-1936	6,636,392
Total ...	40,910,371

2. *Distribution.*—The geographical distribution of the vaccine in India and to places outside India during the years 1935 and 1936 is given below :—

	Number of doses.	
	1935.	1936.
British India.		
Bombay Presidency	113,567	59,833
Madras Presidency	119,919	116,537
Bengal, Bihar and Assam	60,336	20,128
United Provinces of Agra and Oudh...	152,846	156,468
Punjab	71,756	33,540
North-West Frontier Provinces	15	79
Central Provinces and Berar	33,179	22,624
Delhi Province	.....	193
Sind	.....	2,840
Burma	131,748	193,120
	683,366	605,362
Indian States.		
Southern Maratha States	5,152	6,483
Hyderabad Deccan	214,373	154,000
Mysore	81,008	97,648
Patiala	6,416	784
Jammu	1,995	5,040
Jaipur	8,050	5
Jodhpur	98	36
Cambay	.....	96
Bhopal	1,072	1,216
Indore	.....	864
Cochin	.....	1,968
Rampur	1,600	256
Rewa	260	40
Bharatpur	.....	192
Baroda	13,008	.....
Faridkot	150	.....
	333,182	268,628
Foreign Countries.		
China	.....	592
Ceylon	.....	152
Aden	.....	5,040
Port Blair	.....	32
Federated Malay States	384	250
Basra	112	.....
Cairo, Egypt	.....	1,000
	496	7,066
Total number of doses ...	1,017,044	881,056

B. *Cholera Vaccine.*

Up to 1933, the Institute manufactured only plague vaccine, but in accordance with its new policy of making itself as helpful as possible, within its limited resources, to the Health authorities of the Presidency, it took up the manufacture of cholera vaccine on a large scale as an experimental measure without asking for additional personnel for the purpose. When the manufacture was begun a demand of about 50,000 doses of cholera vaccine a year was expected, but the actual demand has far exceeded this figure; during the year under review the demand reached a figure of over 700,000 doses. The extra work involved has proved too much for the limited staff of the Institute.

The table below gives figures for the issue of doses of cholera vaccine month by month during the last three years :—

Cholera Vaccine issued month by month from 1934 to 1936.

—	1934.	1935.	1936.
January	1,812	2,070	11,191
February	540	4,439	12,801
March	68	4,945	4,553
April	2,358	3,305	4,311
May	1,660	7,227	21,364
June	2,131	8,110	192,563
July	7,135	91,970	118,183
August	67,050	127,112	92,487
September	34,465	94,259	64,173
October	49,263	24,634	55,370
November	11,287	19,430	33,330
December	3,308	5,530	95,265
	181,077	393,031	705,621

C. *Other Vaccines.*

At about the same time and in pursuance of the same policy the manufacture of Meningococcal Vaccine and T. A. B. vaccine was also taken up.

The figures for these two vaccines are given in the two tables below :—

Meningococcal Vaccine.

—	1934.	1935.	1936.
January	.....	90	5,908
February	.....	650	2,445
March	.....	5,110	1,943
April	46	4,550	3,606
May	1,171	920	1,350
June	90	580	520
July	1,575	225	110
August	1,650	9	207
September	6,150	.....	278
October	3,025	645	9
November	2,300	250	.....
December	1,510	8,055	5
Total ...	17,517	21,084	16,381

T. A. B. Vaccine.

—	1934.	1935.	1936.
January	27	78	198
February	405	243	571
March	565	78	437
April	215	223	1,162
May	13	374	551
June	270	392	179
July	138	298	236
August	771	324	1,398
September	879	546	446
October	357	436	321
November	103	285	130
December	74	268	117
Total ...	3,817	3,545	5,746

D. *Sera.*

To ensure a supply of reliable sera to the Government hospitals in the Presidency, Government authorised the Institute to make the necessary arrangements from the middle of the year under review. Through the courtesy of Dr. Th. Madsen, the Institute has been able to get sera in bulk from the State Serum Institute, Copenhagen. This serum institute occupies a very prominent position among the serum producing institutions of the world and ensures a supply of reliable sera at favourable prices. Sera were obtained in bulk and were bottled at the Institute to make the cost to the hospitals still less.

SECTION II.

Epidemiology of Plague.

The Department of Plague Epidemiology under Rao Bahadur G. D. Chitre, in addition to the research work in connection with the relative immunity of rat population in different parts of India—reported in Part II of this Report, carried out the following duties :—

A.—Training of Health Personnel.

Requests are constantly received from various Health organisations for the training of health personnel in plague epidemiological and plague preventive work. During the year the following men took training in this Department :—

1. Dr. W. K. Chen, M.D., a member of the Chinese National Quarantine Service was sent to the Institute by the League of Nations Health Section. Studied in the Department for 3 months.

2. Dr. S. H. Kamat, M.B.B.S., D.Hy. (Bombay) of the Presidency Health Department was trained in HCN fumigation methods.

3. Dr. P. Mahomed Ali, Health Officer of Cochin Government, received training for five weeks at the request of his Government.

4. Ten students of the Sanitary Inspectors' Class, Bombay, were given a special course of training for a month at the request of the Executive Health Officer of the Bombay Corporation.

5. Twenty students of the College of Sociology received a short course in general plague preventive measures.

6. The 1936 Class of Sanitary Inspectors divided into four batches was given a short course of demonstrations and lectures on plague preventive measures.

B.—Technical Assistance.

Technical Assistance is freely given by this Department to Health authorities of all parts of British India and Indian States, by answering enquiries in connection with the Epidemiology of Plague.

C.—Examination of Bombay City Rats.

The examination of rats and other rodents of Bombay City, sent by the Executive Health Officer of the Bombay Municipality for evidence of plague, was continued during the year. The Municipality maintains a permanent establishment for catching rats and about 2,000 rats are caught every night in the City and they are marked with the localities from where they are caught. Out of these rats those which arrive at the Institute dead are post-mortemed and examined for signs of plague. In doubtful cases smears and cultures are made. This study besides keeping the Municipality informed of any plague epizootic in any particular

part of the town is providing valuable information on the subject of plague epidemiology.

Since the middle of this year, all the rodents are also examined with a view to their classification. So far a note had been made regarding the total numbers of *Rattus rattus* and *Rattus norvegicus* only. But in future *Gunomys varius* (small field rat) will also be enumerated. Some researches in epidemiology have disclosed the fact that these rodents are also intimately connected with the spread of plague.

EXAMINATION OF CITY RATS FOR PLAGUE.

TABLE I.

Rats received at the Institute from the Municipality during 1936.

Months.	Alive.	Dead.	Total.
January	41,905	28,573	70,478
February	32,582	27,757	60,339
March	32,618	27,415	60,033
April	30,403	28,843	59,246
May	30,674	28,546	59,220
June	28,524	25,186	53,710
July	30,910	23,490	54,400
August	34,924	26,551	61,475
September	38,548	25,519	64,067
October	29,862	14,918	44,780
November	28,822	16,345	45,167
December	34,072	19,003	53,075
	393,844	292,146	685,990

TABLE II.

Examination of dead rats for plague during 1936.

Months.	Dead rats examined.	Found plague infected.	
		Rattus norvegicus.	Rattus rattus.
January	16,752	Nil.	Nil.
February	19,925		
March	16,414		
April	17,964		
May	19,917		
June	15,487		
July	14,790		
August	14,343		
September	14,717		
October	8,508		
November	14,905		
December.	9,005		
	182,727	.....	.....

TABLE III.

Relation of human plague deaths to plague deaths among rats during 1936.

Months.	Human plague deaths.	Rats examined.	Plague-infected rats.
January	1*	16,752	0
February	0	19,925	0
March	0	16,414	0
April	0	17,964	0
May	0	19,917	0
June	1†	15,487	0
July	0	14,790	0
August	0	14,343	0
September	0	14,717	0
October	0	8,508	0
November	0	14,905	0
December	0	9,005	0
	2	182,727	.....

* Reported as an imported case.

† Not bacteriologically confirmed.

D. *Examination of Rats from Steamers in the Bombay Harbour.*

The Health Officer of the Port of Bombay forwarded 550 rats (404 killed and 146 alive) from 52 steamers. Out of the former 227 (56·2 per cent.) were *Rattus rattus*, 18 (4·4 per cent.) were *Rattus norvegicus* and 159 (39.4 per cent.) were *Mus musculis*. Amongst 146 live rodents, 110 (75·3 per cent.) were *Rattus rattus* 2 (1·4 per cent.) were *Rattus norvegicus* and the remaining 34 (23·3 per cent.) *Mus musculis* The flea count was done on 143 of the living rodents. These yielded altogether 21 fleas, namely an average of 0·1 flea per rat. Eighteen out of these 21 fleas were *Xenopsylla cheopis* (5 males and 13 females) and the remaining three, *Xenopsylla astia* (1 male and 2 females). None of the above rats was found plague infected.

This investigation is part of a survey started some years ago to see if there was any correlation between the rodent population of the Docks and that of the steamers in the Harbour.

E. *Preparation and Supply of Exhibits on Plague Preventive Measures.*

The Department prepared several exhibits during the year and supplied them to various health bodies and for teaching students of medicine.

F. *The Manufacture of Approved Rat Traps.*

Some years ago a rat trap best suited to conditions in India was designed at the Institute. Ever since then efforts have been made to see that health organisations get their supplies of rat traps true to the specifications laid down. With this object in view private manufacturers are supplied with scale designs and are instructed in testing the trueness of traps. During the year the following institutions and firms were assisted :—

(*a*) Mr. A. A. Patel, Junior Jailor, Central Jail, Nasik Road.

(*b*) Mr. Tyabally Sultanjee, Barbum Building, Bombay No. 3.

(*c*) Mr. M. Ganghadar, L.M.E., Proprietor, Bombay Engineering Bureau, Khetwadi, Bombay.

(*d*) The Frontier Tin and Iron Works, Jullundur Cantonment.

So far the small private firms have proved unsatisfactory. The Institute, therefore, welcomes the fact that Nasik Jail, at the request of the Director of Public Health, has taken up the manufacture of rat traps. It is hoped this will prove to be a reliable source of supply of rat traps.

G. *Isolation of an Organism from Rats showing Lesions like those of Plague.*

In the last report a reference was made to the fact that though no city rats were found to be plague infected, a few rats did show lesions very similar to plague infection. This phenomenon was studied further during the year.

Forty-two strains were obtained from Bombay City rats which showed such signs and were picked up during the year while carrying out the routine rat examination by the rapid method. The results may be summarised as follows :—

Morphology (smears from tissues).

(*a*) When stained by Carbol thionin—

Rods, occurring singly, in pairs or short chains. Majority somewhat square ended, but some with rounded ends, ovoid in shape, resembling *Past. pestis* to a remarkable degree. Some strains show a well-marked unstained region around the bacillus several times the size of the latter, somewhat resembling a capsule.

(*b*) When stained by Jensen's modification of the Gram stain—

Almost all are found Gram positive but occasionally Gram negative and transitional forms are also present.

The organism is a spore-bearer and is motile.

Growth in Liquid Media.

Fragile pellicle, medium becoming clear by sedimentation.

Agar Colonies (two types of colonies).

(1) Round, regular, moist, opaque, slightly raised, glistening, white. The surface of the colony resembles crepe or crumpled tissue paper. The majority of the colonies show well-marked dense centres, more wrinkled than the surrounding thin areas. These dense centres become translucent with age.

(2) Spreading irregular, flat, showing no differentiation in centre and periphery, otherwise similar to No. 1.

Both types of colonies show well-marked hæmolysis on blood agar.

Carbohydrate reactions.

Arabinose	(−).	Starch—Hydrolysed.	
Xylose	(−).	Inulin	(−).
Rhamnose	(−).	Dextrin	(+).
Glucose	(+).	Adonite	(−).
Lactose	(−).	Mannite	(−).
Saccharose	(+) (late 3-7 days).	Dulcit	(−).
Maltose	(+).		
Raffinose	(−).		

Litmus Milk.

No clot. Peptonized to an amber coloured liquid, peptonisation beginning from top.

Potato.

Profuse whitish growth.

Protein reactions.

Indol (−).
V. P. (−).
M. R. (−).
H_2S. (−).
Gelatin—Liquefied.
Nitrates—Reduced.

Animal Inoculation.

Like plague the organism is a cutaneous passer in the case of rats.

(−) = No acid or gas.
(+) = Acid, no gas.

The organism seems to be either *B. megatherium* or *B. ruminatus*, a closely allied organism. A strain of *B. megatherium* obtained from the Lister Institute gave the same carbohydrate and protein reactions as stated above and even similar colonies.

Further work on the serology of the organism is in progress.

SECTION III.

Department of Bio-Chemistry.

1. *Nutritional Work.*—Work to ascertain the conditions under which the Institute strain of white rats is suitable for the biological assay of the two principal fat-soluble vitamins A and D has been continued. The details are recorded elsewhere in this report.

2. *Concentration of Anti-plague Serum.*—During the year 18 litres of horse serum have been concentrated by the sodium sulphate globulin precipitation method. The technique followed was the same as had been described in the previous report of this Institute (page 26, Report of the Haffkine Institute, 1932-35).

3. *Normal Clinical Standards for Indians.*—The work of determining the various constituents of the blood to establish standards for normal Indian adults has been continued. Thirty-nine normal subjects between the ages of 20 and 30 years were examined during the year under report.

We have been able to obtain data for over one hundred adult young men. The results of this investigation will shortly be published.

The following table gives the results obtained by the examination of the thirty-nine subjects done this year :—

Table giving data for normal bloods.

Constituent.	Method.	Range.	Average.
Non-protein Nitrogen.	Folin Micro.	16·21—21·02 mg. per cent. ...	18·87 mg. per cent.
Urea Nitrogen ...	Do. ...	10·69—14·13 „ „ „ ...	12·89 „ „ „
Uric Acid ...	Do. ...	2·07—3·74 „ „ „ ...	3·00 „ „ „
Creatinine ...	Do. ...	1·04—1·83 „ „ „ ...	1·40 „ „ „
Total Creatinine .	Do. ...	2·19—3·68 „ „ „ ...	3·02 „ „ „
Amino Acid Nitrogen.	Do. ..	2·71—4·61 „ „ „ ...	3·72 „ „ „
Chlorides as NaCl.	Whitehorn...	347·5—470·0 „ „ „ ...	415·5 „ „ „
Cholesterol ...	Leiboff ...	153—224 „ „ „ ...	187·7 „ „ „
Inorganic Phosphorus.	Fiske and Subbarow.	2·93—3·99 „ „ „ ...	3·52 „ „ „
Serum Calcium...	Clarke and Collip.	9·02—11·02 „ „ „ ...	9·68 „ „ „
Sugar ...	Folin Micro.	68·5—102·6 „ „ „ ...	76·8 „ „ „
Fibrin ...	Wu and Ling.	0·16—0·27 g. per cent. ...	0·22 g. per cent.
Albumin ...	Do. ...	3·87—4·68 „ „ „ ...	4·24 „ „ „
Globulin ...	Do. ...	1·73—2·50 „ „ „ ...	1·95 „ „ „

4. *Shrinkage of red blood cells due to potassium oxalate.*—The use of neutral potassium oxalate as an anti-coagulant in blood causes a shrinkage of the red blood cells, and consequently the value obtained for the volume of packed cells in bloods where potassium oxalate is used as an anti-coagulant is not real. Different values for the shrinkage are given in literature by different workers. Experiments were done to determine the value for shrinkage caused by neutral potassium oxalate (2 mg. per c.c. of blood). Oxalated blood was centrifuged against samples of the same blood to which an isotonic anti-coagulant, heparin or hirudin, had been added. It is assumed that neither heparin nor hirudin causes any contraction of the size of the red cell. A speed of 4,500 revolutions per minute for thirty minutes was used. The average shrinkage value obtained by experiments done on ten different samples of blood was 5·71 per cent. and 5·79 per cent. of the volume of packed cells in hirudinised and heparinised blood respectively, the difference being within experimental error.

The detailed results of this investigation are given elsewhere in Part II of this report and will also be published shortly.

5. *Estimation of Ascorbic acid content in the blood and urine of normal Indians.*—In the year under report forty-two normal young men were examined for the ascorbic acid content in their blood. The urines of only eight subjects were examined. The work is being continued. The results obtained are given in Part II of this report.

6. *Relation between coagulation time and the concentration of Russell's Viper Venom.*—A few experiments done with different concentrations of the venom show that the time required for coagulating fowl's plasma is not proportional to the concentration of the venom. Work is being continued.

7. *Chemical Analyses.*—The Unit has continued to be increasingly made use of by hospitals and private practitioners for various biochemical examinations. The following table gives the work done during the year under report.

Routine Examinations carried out in 1936.

Blood—		*C. S. Fluid*—	
Non-protein Nitrogen ...	64	Total Protein ...	13
Urea Nitrogen ...	68	Globulin ...	10
Uric Acid ...	7	Chlorides ...	12
Creatinine ...	14	Sugar	10
Sugar ...	27	Non-protein Nitrogen...	1
Chlorides ...	2	Cholesterol ...	2
Cholesterol ...	26	Lange's Colloidal Gold Test	27
Phosphorus ...	1		75
Serum calcium ...	10	*Fæces*—	
Hæmoglobin ...	17	Fat	2
Cell Volume ...	15	Detection of lead ...	1
Indices ...	15	Occult blood ...	5
Vanden Bergh Reaction .	9		8
	275		

Urine—		*Miscellaneous—*	
General qualitative test.	27		
Total Nitrogen ...	7		
Urea Nitrogen .	4	Glucose Tolerance Test.	9
Uric Acid ...	6	Coagulation Time of blood.	1
Sugar ...	28	Fragility of erythrocytes.	6
Chlorides ...	2	Kidney Function test...	25
Albumin ...	8	Gastric analysis ...	15
Urobiten ...	1	Basal metabolism ...	26
Indican ...	2	Fluid from Abdominal cavity.	1
Blood ...	6		
Bile pigments ...	4		
Ammonia Nitrogen ...	1		
Detection of lead ...	4		
Urinary calculus ...	1		83
	101	Total ...	542

SECTION IV.

Department of Pharmacology.

The scope of the activities of the department was enlarged during the year under review. Besides the research work carried out (detailed in Part II of this report) arrangements were made to conduct biological assay of certain drugs. We can now undertake assays of Digitalis, Squill, Strophanthus and Ergot. We hope to extend the field for biological assays further and undertake biological assays of other drugs like extracts of suprarenal medulla, the posterior pituitary gland, etc.

Mr. H S. Mahal, a Tata Research Scholar was admitted to the department in March 1936 to conduct pharmacological investigations on some chemicals he had prepared synthetically. He investigated their pharmacological action with special reference to their anthelminthic properties and arrived at the conclusion that these chemicals which are flavones, do not possess any anthelminthic property. Mr. Mahal has also collaborated in the work on "Choline esterase". A summary of this work is given in Part II of this report.

The pharmacological action of plasmoquine was investigated during the period under review. It was found that this drug did not reduce temperature by itself but in combination with quinine it reduced temperature more quickly than quinine alone. The drug given in larger doses produced irregularity of the heart and a fall in blood pressure. The most important action on blood was that of formation of methæmoglobin which is the cause of cyanosis, sometimes seen after administration of plasmoquine. The liver of guinea-pigs was very frequently damaged after administration of toxic doses of the drug to these animals.

The action of plasmoquine with special reference to pregnancy is now under investigation. The object is to investigate whether it is safe to give plasmoquine during pregnancy or not. Some preliminary work has already been done and it has been found that administration of even toxic doses of plasmoquine does not produce abortion in pregnant animals. These preliminary tests indicate that plasmoquine is tolerated fairly well and is not contraindicated in the treatment of Malaria during pregnancy.

Investigations on the action of acetylcholine have been continued. It is now generally agreed that the effects of stimulation of nerves are brought about by the liberation of chemical substances at the periphery and acetylcholine has been proved to be the chemical substance concerned in the case of a large number of nerves. Whether this chemical transmission holds good in the case of the central nervous system is a doubtful problem. Attempts to demonstrate liberation of acetylcholine in the central nervous system and in the cerebrospinal fluid after stimulation of afferent nerves have been made but the experiments do not justify a conclusion that such a stimulation leads to liberation of acetylcholine.

The problem of the action of acetylcholine has also been investigated by studying the activity of the specific enzyme which destroys acetylcholine. Estimations of the acetylcholine content of the brain and the enzyme in the blood, of different species of animals showed that a direct relationship exists between these two. If the acetylcholine content of the brain was high, the enzyme concentration in the blood was also found to be high and *vice versa.* This enzyme which destroys acetylcholine has been investigated further and experiments made to see if the enzyme concentration in the blood is altered in different physiological conditions. It has been found that changes in temperature and diet do not modify the enzyme concentration, nor does rest or exercise. Further work on this subject is in progress.

Biological Assay of Drugs.—As has been mentioned in the beginning this Laboratory now accepts drugs for biological standardisation. Standards which are maintained in the National Institute for Medical Research, Hampstead, London, have been obtained and standards established for this Laboratory. Some firms have taken advantage of this opportunity and have sent their preparations for standardisations here. It should be a matter of concern to all medical men that some of the products which are on the markets are of remarkably low potency. For example, a sample of Tincture Digitalis assayed was only 25 per cent. of the standard and another sample of Tincture Strophanthus had a potency merely 1/10th of what it should actually possess. These samples were obtained from manufacturers soon after their preparation and therefore deterioration in potency due to unsuitable conditions of storage, etc., was not a factor involved. The potency of these preparations after they have been badly stored in chemists' shops must fall still lower.

Snake-bite Remedies.—We receive quite a large number of drugs as "Snake-bite Cures" from various sources all over India. Usually we find the same drugs are sent to us over and over again for tests. No drugs have been found to possess any preventive or curative properties against Cobra or Viper venoms. We have now restricted our testing of snake-bite cures only to those drugs which are sent by responsible persons. About six such "remedies" were tried experimentally and were found to be without any effect in Cobra or Viper poisoning.

SECTION V.

DEPARTMENT OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY.

A. Chemical Work.

Waters.—During the year under report 18 samples of water were examined; majority of these being for sanitary purposes. The chemical composition of these waters showed extreme variations in total solids and chlorides as compared to the recognised chemical standards of purity.

B. Bacteriological Work.

Waters.—During the year under review the following samples were examined.

Drinking waters	33
Drinking waters examined for isolation of specific pathogenic organisms or for testing various sterilising methods.	9
Sewages and effluents	10
Waters from Swimming Baths	2
Total ...	54

For drinking waters the method outlined in Report No. 71 of the Ministry of Health on the Bacteriological Examination of Waters was followed.

C. Miscellaneous Work.

Two samples of shell fish were examined to test their suitability for human consumption.

Two consignments of plants imported from abroad were fumigated with hydrocyanic acid gas. This work was undertaken on behalf of the Customs Department.

Stains and standard solutions were prepared and supplied to the various laboratories in this Presidency.

SECTION VI.

Diagnostic Department.

A. Bacteriological Section.

The routine work carried out is summarised in the accompanying table.

Material examined.	Positive.	Negative.	Total.
A. Blood—			
(*a*) Films for microscopical examination—			
for malaria ...	63	109	172
„ filaria ...	.....	1	1
„ relapsing fever ...	.....	2	2
„ differential leucocytic count ...	.....	.....	35
(*b*) Count for R. B. Cs. ...	.....	.....	14
„ „ W. B. Cs. ...	.....	.....	12
(*c*) Cultures for organisms (B. Typhosus, etc.)	8	4	12
(*d*) Serological examinations—			
Wassermann Reaction ...	960	2,636	196 (Indefinite.) 3,792
Kahn Reaction ...	162	424	25 (Indefinite.) 611
Blood grouping	.....	.....	7
Agglutination tests—			
for enteric group ...	43	129	172
„ undulating fever ...	1	2	3
„ Weil Felix reaction ...	.....	2	2
„ B. coli ...	.....	1	1
„ B. dysentery ...	1	1	2
B. Sputum—			
Microscopical examination for tuberculosis...	9	16	25
Cultural examination for various micro-orgamisms	3	0	3
C. Urine—			
General Analysis ...	.....	.....	212
Cultural Examination ...	151	10	161
Examination—miscroscopical and animal experiments—			
for Tuberculosis	4	8	12
„ Bilharzia hæmatobia	2	5	7
„ Aschheim-Zondek Test	2	1	3
D. Fæces—			
(*a*) Microscopical examination for ova, and amobæ	101	143	244
(*b*) Cultural examination—			
for Cholera, Typhoid and Dysentery ...	7	43	50
for miscellaneous organisms ...	.....	.....	18
(*c*) Microscopical and animal experiments for T. B.	.....	1	1
(*d*) Examination for occult blood ...	2	1	3

Material examined.	Positive.	Negative.	Total.
E. C. S. Fluid—			
(*a*) Microscopical examination for T. B. and other organisms	2	10	12
(*b*) Cell Count	.....	.....	20
(*c*) Cultural examination	1	10	11
F. Pus—			
Microscopical and cultural examination ...	2	3	5
G. Miscellaneous microscopical examination of smears—			
Smears for B. Diphtheria	1	3	4
„ „ Gonococcus	4	6	10
„ „ Leprosy	2	2	4
„ „ Meningococcus	1	11	12
„ „ Spirillum of rat-bite fever ...	.....	1	1
„ „ Tuberculosis, from pleuritic and other cyst fluids	1	3	4
H. Miscellaneous cultural examination—			
Swabs for B. diphtheria	3	6	9
„ „ Meningoccus	.....	8	8
I. Miscellaneous animal tests—			
B. diphtheria	1	.....	1
J. Miscellaneous work—			
Disinfectants for carbolic cœfficients ...	...	...	1
Identification of snakes ...	...	...	12
Stock cultures (subcultured at suitable intervals).	...	...	327
K. Supplies—			
(1) *Vaccines*:			
(*a*) autogenous vaccines	...	...	42
(*b*) stock staphylococcus and streptococcus vaccines.	...	...	6 doses.
(*c*) B. coli communis vaccine ...	...	...	12 do.
(*d*) killed bacterial emulsions:			
B. typhosus ...	...	...	1633 c. cs.
B. paratyphosus A ...	...	...	1455 c. cs.
Do. B ...	...	...	1396 c. cs.
Do. C ...		...	33 c. cs.
M. melitensis ...	...	...	46 c. cs.
B. abortus ...	...	...	29 c. cs.
B. coli ...	...	...	46 c. cs.
B. dysenteriæ Shiga ...	...	...	23 c. cs.
Do. Flexner ...	...	...	20 c. cs.
(2) *Sera*:			
Group sera	...	...	33 c. cs.
(3) Living cultures of pathogenic microorganisms.	...	...	40
(4) Media—			
Lœffler's serum agar tubes ...	...	...	304
Blood agar tubes	...	...	925

B. Pathological Section.

A large number of pathological tissues were received and examined during the year under review. As compared with last year there was an increase of 35 per cent. This was due to increase in the number of examinations carried out for both routine diagnosis and research purposes. The following table shows the total number of pathological tissues received and examined during 1936 :—

—	1935.		1936.	
	No.	Per cent.	No.	Per cent.
Non-malignant tumours ...	57	8·9	51	6·4
Malignant tumours ...	55	8·5	86	10·6
Tissues found to be normal ...	3	0·5	3	0·4
Miscellaneous pathological conditions (including research specimens).	309	48·1	441	55·0
Received in a state unfit for examination.	6	0·9	3	0·4
Animal brains for Negri bodies...	213	33·1	218	27·2
Total ...	643	.....	802	.....

The following case is worthy of note.—A Hindu male aged 26 was admitted for "swellings" in different parts of the body, i.e., right shoulder, cheek, thigh, etc. The tissues were removed from all these sites. One piece from the upper part of right leg was sent for histological examination with the remarks that the tissue was "lobulated, capsuled, subcutaneous, easily ennucleated, no infiltration, pockets of pus in a part of the tumour-fatty tissue ?".

Histological examination revealed a typical picture of Rhinosporidiosis. Five months later another tissue was removed from the inner aspect of right ankle. This was similar to the previous tumour and on section showed a typical picture of Rhinosporidiosis.

This is recorded here as a case of Rhinosporidiosis occurring in a multiple form at most unusual sites.

Malignant Tumours.—Of the 86 specimens subjected to histological examination 44 were carcinomas, 19 sarcomas, 3 rodent ulcers, 18 epitheliomas, 1 embryoma of testis and 1 hæmangioendothelioma. The last interesting specimen was obtained from an infant 1 year of age and occurred as a small tumour on the back in the middle line about the level of the junction of lower lumbar vertebra and the upper part of the sacrum.

50 per cent. of epitheliomas and 40 per cent. of carcinomas occurred in Female Reproductive Organs, cervix of the uterus being the commonest site. 10 cases of carcinoma of the breast were diagnosed. Rodent ulcers were confined to the face.

Histo-pathology of Plague.—Experimental studies in pathology of plague in rodents were undertaken to trace the exact path of infection and various pathological changes that result from experimental infection. The studies so far made have been in white mice and white rats. The work is proceeding.

SNAKES AND SNAKE VENOM.

Snakes.

The following snakes were received during the year 1936 :—

Cobras	41
Russell's Vipers	84
Kraits	12
Echis carinata	308

Venom Supplies.

Cobra	60·1 gms.
Russell's Viper	60·924 gms.
Echis carinata	3·5 gms.

SECTION VII.

ANTIRABIC DEPARTMENT.

This department under the charge of Military Assistant Surgeon, H. Maurice, I.S.O., I.M.D., has continued to grow. During the year under review 2,961 persons applied for antirabic treatment at the Institute. Of these, 2,331 persons completed the full course of treatment; 622 were advice cases, as the biting animals which were under observation were alive and well for 10 days after having inflicted the bite and found to be not rabid; and 8 persons discontinued treatment. To-date only 2 persons developed hydrophobia after treatment giving a mortality rate of 0·08 per cent.

Outcentres.—Five new antirabic centres were opened during the year bringing the total of outcentres to 69 and there is a likelihood of more being opened in the near future. To take charge of the newly opened centres five medical officers underwent training in antirabic treatment at the institute.

During the year 5,259 antirabic treatments were issued to these centres. These figures include 319 treatments issued for veterinary use.

As the health returns will not be ready before July next it is not possible to give the mortality rate and the statistical table for the current year, but statistics for the previous 12 months are given at the end of this report.

The details of the treatment carried out at the institute for 1935 and 1936 are summarised as below :—

	1935.	1936.
Number of persons applied for treatment .	2,955	2,961
Advice Cases	657	622
Those completed full treatment ...	2,274	2,331
Percentage of Health Returns ...	96·7%	Awaited.
Cases died of Hydrophobia	4	,,
Total Mortality Rate	0·17%	,,

Table showing the number of brains received and examined at the Institute for evidence of Rabies during the year 1936 :—

—	Number.	Percentage.
Received in a state unfit for examination	4	1·8
No evidence of Rabies	44	20·2
Positive to Rabies " Negri Bodies present "	170	78·0
Total ...	218	.....

Statement showing the classification of different animals with the result of their brain examinations for Rabies :—

—	Positive.	Negative.	Un-suitable for Exm.	Total.
Dogs	165	41	4	210
Jackals	...	1	...	1
Monkey	...	1	...	1
Cow calves	3	...	...	3
Buffaloes	1	1	...	2
Human being	1	...	...	1
	170	44	4	218

Statement showing the classification of brains and different places from where the brains were received :—

—	Positive.	Negative.	Unsuitable for Exm.	Total.
Received through the Bombay Veterinary College.	54	10	...	64
Received through the Bombay Police.	12	1	...	13
Received through the Bombay private owners.	2	1	...	3
Received through other places in Bombay.	2	5	...	7
Received through out-stations.	100	27	4	131
	170	44	4	218

SECTION VIII.

Income and Expenditure.

The following table shows the income and expenditure of the Haffkine Institute for the year 1936-37 :—

Receipts.	Rs.	Expenditure.	Rs.
XXIII—Public Health—		33-D—Public Health—	
		D—Bacteriological Laboratories—	
		Haffkine Institute—	
Sale proceeds of Sera and Vaccines etc.	3,02,227	Pay of Officers ...	72,080
Miscellaneous ...	1,590	Pay of Establishment ...	76 822
		Allowances, Honoraria etc.	9,204
		Contingencies ...	1,36,114
		Total ...	2,94,220
		33-E—Public Health—	
		E—Pasteur Institutes—	
		Pay of Officers ...	13,200
		Pay of Establishment ...	4,318
		Allowances, Honoraria etc.	332
		Total ...	17,850
Total Receipts ...	3,03,817	Total Expenditure ...	3,12,070

PART II.

RESEARCH WORK.

I. Plague Vaccine.

(SOKHEY.)

Using the standardise quantitative methods for measuring the virulence of *Past. pestis* cultures and for measuring the protective value of plague vaccines described in the last report (1932-35), the study of plague vaccine during the year, was continued.*

A. The Relative Protective Power of Broth Vaccines incubated at 27°C. and those incubated at 37°C.

In the previous report we gave results of experiments with one vaccine: these were

Broth Vaccines.	Minimum Mouse Protective Dose:
Vaccine 18B, incubated at 27°C. for 4 weeks (4 Experiments)	0·002 c.c.
Vaccine 18B, incubated at 37°C. (4 Experiments)	0·004 c.c.

During the year we tested two more vaccines with the following results:

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* Regarding the method it should be added here that protocols were submitted to Professor Major Greenwood, London School of Hygiene and Tropical Medicine. Through his courtesy, Dr. J. O. Irwin very kindly statistically evaluated the results. Taking the protocol published on page 61 of Report of the Haffkine Institute for the years 1932-35 and using the method explained in M. R. C. Report No. 183, 1933, he drew up a report. In brief his findings are:—

$$\left.\begin{array}{l} \text{L.D. } 50 = \bar{3}{\cdot}2141 \\ \text{L.D. } 60 = \bar{3}{\cdot}4374 \end{array}\right\} \text{Standard Error} = {\cdot}1149$$

Median Protective Dose	= 0·00164	95 per cent. Limits. 0·00097—0·00278
60 per cent. Protective Dose	= 0·00274	0·00161—0·00465

and he observed "that the limits are respectively 1·70 times and 1/1·70 times the estimates. It might well be required to make a more accurate determination than this which could only be done by increasing the number of animals used. 4 times the number of animals would about half the standard error and reduce the ratio to about 1·30, 10 times the number of animals would only bring it down to 1·18." It was not practical for us to use a larger number of animals, as even at present we use 25 animals for each experiment and usually repeat the experiment 4 times making 100 animals for each test. Even with the number of animals used the error is limited and its limits well defined.

Broth Vaccines.	Minimum Mouse Protective Dose.
Vaccine 125H (15th September 1936), incubated at 27°C. for 4 weeks (4 Experiments) ...	0·002 c.c.
Vaccine 125H (15th September 1936), incubated at 37°C. for 4 weeks (4 Experiments) ...	0·015 c.c.
Vaccine I (21st October 1936) incubated at 27°C. for 4 weeks (2 Experiments) ...	0·002 c.c.
Vaccine I (21st October 1936) incubated at 37°C. for 4 weeks (2 Experiments)	0·004 c.c.

In all cases growths were killed at 55°C. for 15 minutes and then carbolised to contain 0·5 per cent. phenol. These experiments show that broth vaccines incubated at 27°C. are more potent than those incubated at 37°C.

B. *The Relative Protective Power of Agar Vaccines incubated at 27°C. and those incubated at 37°C.*

For these experiments a 48-hour broth culture of a given strain was planted on nutrient agar slopes. These were incubated for 48 hours at 27°C. and some at 37°C. Suspensions were then made with saline and standardised to contain 1500 million organisms per c.c. Growths were killed at 55°C. for 15 minutes and then phenol added (0·5 per cent.). The number of 1,500 millions organisms was fixed by actual experiment, so that the best agar vaccine may have about the same protective power as the best broth vaccine.

Agar Vaccines.	Minimum Mouse Protective Dose.
Vaccine 125H (24th August 1936), incubated at 27°C. (4 Experiments)	0·1 c.c.
Vaccine 125H (24th August 1936) incubated at 37°C. (10 Experiments)	0·002 c.c.
Vaccine I (26th August 1936) incubated at 27°C. (2 Experiments)	0·1 c.c.
Vaccine I (26th August 1936) incubated at 37°C. (2 Experiments)	0·004 c.c.

These results show that agar grown vaccines incubated at 37°C. are more potent than those incubated at 27°C.

Schutze (1932) when he came to the conclusion that vaccines incubated at 37°C. are more potent than those incubated at 27°C. had done part of the work with broth grown vaccines. Our work with broth grown vaccines does not bear out his conclusion. Further

Schutze had put forth the hypothesis, on indirect evidence, that the difference was due to capsular material which developed at 37°C. and not at 27°C. Though agar grown vaccines incubated at 37°C. are markedly more potent than those grown at 27°C., we do not believe that the difference is due to the presence or absence of a capsule. We have been able to stain capsules even when we incubate plague cultures at 5°C. (We have now taken microphotographs and shall publish them shortly). We have made some observations which lead us to believe that the potency of the vaccines depends to a large extent on the number of organisms and their size, i.e., on the bulk of organisms, in the vaccines. There is one more factor involved which we shall discuss lower down. In the case of brothgrown vaccines the growth of organisms is self-limited, and the growth of *Past. pestis* is much less at 37°C. than at 27°C. Hence broth vaccines incubated at 37°C. are poorer than vaccines incubated at 27°C. When we come to agar vaccines, we are in a position to vary at will the number of organisms in a suspension. And it will be noticed that the various suspensions used in these experiments had the number of organisms made equal by actual counts. This factor eliminates the difference operating in the case of broth grown vaccine. But why are agar vaccines made from organisms incubated at 37°C. more potent? Partly the reason for this seems to be that organisms when grown on agar and incubated at 37°C. are much bigger in size than when incubated at 27°C. Thus the actual bulk of organism in the agar vaccines made from cultures incubated at 37°C., is larger than in the vaccines made from cultures incubated at 27°C. though the number is the same. But the difference in bulk is not as great as the potency tests show. There seems to operate another factor also. This will be discussed later.

C. *Live Avirulent Broth Vaccines compared with Heat-killed Broth Vaccines.*

In the previous report we had described a method of maintaining the virulence of plague cultures for long periods, and also a method for making cultures avirulent. We were thus enabled to have the same strain both in a fully virulent and in an avirulent form. Using the virulent and the avirulent forms of Strain 125H, we have made broth vaccines, incubated for 4 weeks. Temperatures of incubation employed were 27°C. and 37°C. The vaccines made from virulent strains were first killed at 55°C. for 15 minutes and then phenol added (0·5 per cent.).

Broth Vaccines.	Minimum Mouse Protective Dose.
Heat-killed Vaccine 125H (15th September 1936) incubated, for 4 weeks at 27°C. (4 Experiments)	0·002 c.c.
Live Avirulent Vaccine 125H (26th September 1936) incubated, for 4 weeks at 27°C. (3 Experiments)	0·008 c.c.

Broth Vaccines.	Minimum Mouse Protective Dose.
Heat-killed Vaccine 125H (15th September 1936), incubated for 4 weeks at 37°C. (4 Experiments)	0·015 c.c.
Live Avirulent Vaccine 125H (26th September 1936), incubated for 4 weeks at 37°C. (3 Experiments)	0·015 c.c.

In the case of broth grown vaccines heat-killed vaccines made from virulent cultures and incubated at 27°C, are superior to live avirulent vaccine grown either at 27°C. or at 37°C.

These results have a marked resemblance to the results of the experiments given above under A and illustrate the same phenomenon. The reason of the poor protective powers of vaccines incubated at 37°C. is very simple, the growth of *Past. pestis* at 37°C. is much less than at 27°C.

D. *Live Avirulent Agar Vaccines compared with Heat-killed Agar Vaccines.*

The strains used in the experiments detailed above under C were used in this experiment. The cultures were grown for 48 hours on nutrient agar slopes and suspensions made in slaine. These were standardised by colony counts to contain 1,500 million organisms per c.c. The virulent cultures were killed at 55°C. for 15 minutes and phenol added (0·5 per cent.)

Agar Vaccines.	Minimum Mouse Protective Dose.
Heat-killed Vaccine 125H (24th August 1936) grown at 27°C. (4 Experiments)	0·1 c.c.
Live Avirulent Vaccine 125H (14th April 1937) grown at 27°C. (2 Experiments)	0·03 c.c.
Heat-killed Vaccine 125H (24th August 1936) grown at 37°C. (10 Experiments) ...	0·002 c.c.
Live Avirulent Vaccine 125H (12th December 1936) grown at 37 c.c. (Preliminary result) ...	0·001 c.c.

Though some of these experiments have still to be repeated, these are not chance results. They are very interesting indeed when compared with the results of broth vaccines made from the same strains. (See C above.) In the case of broth vaccines, heat-killed vaccine incubated at 27°C. proved to be the most potent, and the live avirulent vaccine incubated at 37°C. the least potent. In the case of Agar vaccines made from exactly the same strains, live avirulent vaccines incubated at 37°C. proved to be the most potent and heat-killed vaccine incubated at 27°C. the least potent. A possible hypothesis will be discussed later.

E. *Effect of the method of killing of cultures on the potency of the resultant vaccine.*

In the last report we described an unfinished experiment on the effect of killing a culture at 55°C. for 15 minutes and at 60°C. for 1 hour. (We also reported this experiment in Bull. mens. de l'Off. internat. d'Hyg. pub. 1936, XXVIII, 1078). We were working with agar grown suspensions. We notice that we did not make it clear that we were introducing another factor besides the temperature and duration of killing. The portion of the suspension which was killed at 55°C. for 15 minutes was carbolised after the application of heat, while in the case of other portion of the suspension phenol was added first and then heat applied (60°C. for 1 hour). This was done to conform to the practice of some of the early workers. For instance this procedure was laid down by the Indian Plague Commission. The finished experiment gave the following results :—

Vaccines.	Minimum Mouse Protective Dose.
Vaccine 125H (16th March 1936), killed at 55°C. for 15 minutes and then carbolised (0·5 per cent.) (3000 million organisms per c.c.)	0·002 c.c.*
Vaccine 125H (16th March 1936), carbolised (0·5 per cent.) and then heated at 60°C. for 1 hour (1300 million organisms per c.c.) ...	1·5 c.c.

We have since experimented with growths which were first killed by the application of heat (55°C. for 15 minutes and 60°C. for 1 hour) and phenol (0·5 per cent.) added after the application of heat. In these experiments we have also studied the effect of killing by the addition of 0·5 per cent. phenol only, without the application of heat, and also by the addition of 0·025 per cent. formalin without the application of heat.

Broth Vaccines.

	Minimum Mouse Protective Dose.
Incubated at 27°C. for 4 weeks—	
Vaccine 125-H (15th September 1936), killed at 55°C. for 15 minutes and then added phenol, 0·5 per cent. (4 Experiments)	0·002 c.c.
Vaccine 125-H (15th September 1936), killed at 60°C. for 1 hour and then added phenol, 0·5 per cent. (3 Experiments)	0·015 c.c.
Vaccine 125-H (15th September 1936), killed by the addition of phenol, 0·5 per cent., no heat applied. (2 Experiments)	0·004 c.c.
Vaccine 125-H (15th September 1936), killed by the addition of formalin 0·025 per cent., no heat applied. (2 Experiments) ...	0·008 c.c.

*This result is very different from the result of 125H vaccine (24th August 1936). We have made two more vaccines from this strain and they also give a protective dose of 0·1 cc.

Broth Vaccines.

	Minimum Mouse Protective Dose.
Incubated at 37°C. for 4 weeks—	
Vaccine 125-H (15th September 1936), killed at 55°C. for 15 minutes and then added phenol, 0·5 per cent. (4 Experiments)	0·015 c.c.
Vaccine 125-H (15th September 1936), killed at 60°C. for 1 hour and then added phenol, 0·5 per cent. (2 Experiments)	0·015 c.c.
Vaccine 125-H (15th September 1936), killed by the addition of formalin, 0·025 per cent. (3 Experiments)	0·015 c.c.

Agar Vaccines.

Incubated at 27°C. for 48 hours—	
Vaccine 125-H (24th August 1936), suspension contained 1500 million organisms per c.c., killed at 55°C. for 15 minutes and then added phenol, 0·5 per cent. (4 Experiments) ...	0·1 c.c.
Vaccine 125-H (24th August 1936), suspension contained 1500 million organisms per c.c., killed at 60°C. for 1 hour and then added phenol, 0·5 per cent. (2 Experiments) ...	0·8 c.c.
Vaccine 125-H (24th August 1936), suspension contained 1500 million organisms per c.c., killed by the addition of phenol, 0·5 per cent., no heat applied (2 Experiments) ...	0·2 c.c.
Incubated at 37°C. for 48 hours—	
Vaccine 125-H (24th August 1936), suspension contained 1500 million organisms per c.c., killed at 55°C. for 15 minutes and then added phenol, 0·5 per cent. (10 Experiments) ...	0·002 c.c.
Vaccine 125-H (24th August 1936), suspension contained 1500 million organisms per c.c., killed at 60°C. for 1 hour and then added phenol, 0·5 per cent. (2 Experiments) ...	0·002 c.c.
Vaccine 125-H (24th August 1936), suspensions contained 1500 million organisms per c.c., killed by the addition of formalin, 0·025 per cent., no heat applied. (2 Experiments) ...	0·002 c.c.
Vaccine 125-H (24th August 1936) suspension contained 15 0 million organisms per c.c., killed by the addition of phenol, 0·5 per cent. (3 Experiments)	0·002 c.c.

It will be noticed that in the case of vaccines grown at 37°C. the method of killing does not seem to make any difference while the effect is pronounced in the case of vaccines grown at 27°C. We have repeated these results with vaccines made from another strain, and have obtained similar results. The results do not seem to be chance results.

The phenomenon is being studied for a possible explanation. A clue is offered by an observation reported in the last report, that in the case of broth vaccines, incubated for 4 weeks, the protective power resides almost entirely in the clear liquid portion of the vaccine, i.e., the clear "supernatent fluid" of the vaccine, the debris of the organisms being almost inert. This clearly indicates that the antigenic substance goes into solution during the period of incubation. There is also evidence that during this process of going into solution the protein of the organisms breaks up into a somewhat simpler form. If this breaking up goes beyond a certain point, as in the case of lysis with bacteriophage, the antigenic power is entirely destroyed, as reported in the last report. It appears, therefore, that two factors are involved, the quantity of the bacterial protein and its form. The hypothesis, therefore, emerges that in the vaccines under discussion the antigenic protein (we are ignoring for the present any part played by any carbohydrates) is present in the following forms: (1) Protein in 48 hour agar growths incubated at 27°C., the optimal temperature of growth for *Past. pestis*, (2) Protein in solution in broth growths incubated at 27°C. for 4 weeks, (3) Protein in solution in broth growths incubated at 37°C. for 4 weeks and in agar growths incubated at 37°C for 48 hours. An incubation temperature of 37°C. is very unfavourable for the growth of this organism, and when incubated at this temperature, the growth is very poor and the organism swells up and a number actually break up. It is probable that when incubated at this temperature the protein breaks up a stage further than in 2, both in cases of broth growths and agar growths. To this may be added a fourth form, protein in still simpler form produced by lysis with bacteriophage. The probabality, then, is that protein of Form 1 is the most coagulable one, and the coagulability progressively diminishes from Forms 1 to 4, while protective power increases progressively from Forms 1 to 3 and then disappears.

F. *Effect of Storage on the Potency of Live Avirulent Vaccines.*

It has been our practice to store at 4°C. ± 2 all vaccines we were engaged in testing. It takes about 5 weeks for each test and usually we make as many as four tests and even more on occasions. Working with heat-killed vaccines, we had found that vaccines did not show any measurable loss of potency for months. One vaccine we had tested at about monthly intervals for 12 months and no loss of potency had been detected. But when we came to work with live avirulent agar grown vaccines, we were surprised to find that they deteriorate very rapidly, so rapidly indeed, that it seems to us

they could not be issued out from a central laboratory for use in a big country like India. This fact alone makes the use of live avirulent vaccines an unpractical proposition. We give some results below to illustrate our point.

Live Avirulent vaccine 125H (12th December 1936) suspension for 48 hours growth at 27°C. on agar, 1500 million organisms per c.c. when tested on 15th December 1936 gave a minimum mouse protective dose of 0·05 c.c.

The same vaccine when tested on 10th March 1937, i.e., three months later, it failed to give any protection at all even with as large a dose as 0·6 c.c.

A second vaccine gave almost similar results. We are studying this point further, as it is of extreme importance.

G. *Growth of Past. pestis at different temperatures of incubation and its possible relationship to the start and end of epidemics during certain seasons.*

In the previous report we had reported some preliminary results, but during the year we obtained more accurate incubators and generally perfected our methods and have made extended observations on this subject.

The view held so far has been that "the temperature of growth of the plague bacillus is from 4°C. to 43°C. with optimum at about 30°C."

We are still engaged on this study, but we have sufficient results to show that the optimal temperature of growth is 27°C. and that the growth entirely ceases at 1°C. The most interesting part of our results is that growth at 29°C. is about half of the growth at 27°C. and the growth at 33°C. is about one-third of the growth at 27°C. Similarly growth at 21°C. is half of the growth at 27°C.

The observations are of some importance. It has been repeatedly noticed that epidemics suddenly come to an end with the rise of atmospheric temperature, and also that epidemics do not start until the atmospheric temperatures have reached a certain point. We believe our observations on the growth of *Past. pestis* at different temperatures indicates a possible factor which operates in addition to the other factors noted previously. Flea is poikilothermic and the atmospheric temperature materially affects the multiplication of the organisms in the insect.

H. *Immunisation of different laboratory animals with heat-killed plague vaccines.*

Beginning with Kolle and Otto, a large number of workers including Strong and Rowland have held that guinea-pig could not be immunised with heat-killed vaccines. More recently, Otten (1935) came to the conclusion that as far as the dead vaccines are concerned "the nature of the experimental animal was far more essential to the results".....and that "*no dead vaccine whatever* conferred satisfactory protection to guinea-pigs and wild rats".

We started an investigation into this question employing the new standardised methods. In a previous report we had reported that contrary to the prevailing view we had found it quite easy to confer very good immunity on guinea-pigs, white rats, and wild house rats with heat-killed vaccines. We have continued work on this problem with a view to find the minimum protective dose of our heat-killed vaccine for these animals.

The experiment is not complete yet, but we indicate below how far we have gone.

Taking a heat-killed vaccine with a minimum mouse protective dose of 0·002 c.c., we find that the following doses give high protection to the following animals :—

Wild Rat	(average weight 120 g.)	0·025 c.c.
White Rat	(do. do. 200 g.)	0·025 c.c.
Guinea-pig	(do. do. 360 g.)	0·01 c.c.

These doses are not minimum protective doses, which we believe will be much smaller. The work is proceeding.

II. Antiplague Serum.

(SOKHEY.)

Work on the immunisation of horses as described in the last report has been continued. In order to have enough serum for a sufficiently large field test, we have immunised ten horses and have somewhat improved our methods, with the result that the sera we have recently obtained show a higher potency.

Prophylactic potency tests of the serum produced last year gave a mouse dose of 0·05 c.c., the new serum produced this year gives a mouse dose of 0·025 c.c. We expect much better results from this serum in the treatment of human cases.

Field Tests.—At the time we presented our last report, Dr. Wagle was conducting a field test at Hyderabad, Deccan, and at the time of reporting he had dealt with 34 cases: for these we gave results in the last report. The test was continued and after we had dealt with 50 cases, our serum with a mouse protective dose of 0·05 c.c. was exhausted. We dealt with 20 more cases with a much poorer serum.

For this test, cases were taken in strict rotation: every alternate admission was treated and the others were used as controls. Admissions 1, 3; 5, 7 ..... to 49 were treated with our serum and admissions 2, 4, 6, 8 ..... to 50 were used as controls. No selection of any kind was made except that cases moribund at the time of admission were excluded from both sides. They died within a few hours of admission. Such cases were few. The cases used as controls received the usual hospital treatment in vogue for plague cases. All cases were classified according to the degree of

septicæmia present at the time of admission, as recommended by the Indian Plague Commission. Blood was drawn from a vein of each case and 0·25 c.c. was spread on an agar slope and incubated for 48 hours and colonies counted, and cases divided into the following four groups :—

1. Blood sterile in 0·25 c.c. 0
2. 1 to 10 colonies from 0·25 c.c. ... ... +
3. 11 to 100 colonies from 0·25 c.c. ... ... ++
4. More than 100 colonies from 0·25 c.c. ... +++

Similar cultures were taken, as far as possible, from each patient every day till the patient either died or recovered. These examinations gave us a very good insight into the progress of the disease. It was noticed in control case that whether a case on the day of admission had any septicæmia or not, if it proved fatal it always developed septicæmia before death. In the case of patients treated with the serum, the blood became sterile if the patient proceeded to recovery : in one case death took place even after blood had become sterile. This was due to streptococcal cellulitis. Ordinarily serum was given in 20 c.c. doses and repeated as indicated by the condition of the case. The total quantity used per case varied roughly from 60 c.c. to 160 c.c. We give below a summary of cases grouped by the degree of septicæmia at the time of admission, treated with serum with a mouse protective dose of 0·05 c.c.:—

Classification.	Serum.		Controls.	
	Number.	Deaths.	Number.	Deaths.
0	15	0	16	5
+	6	2	5	5
++	1	1	1	1
+++	3	2*	3	3
Total ...	25	5 (20 per cent.).	25	14 (56 per cent.).

*One patient died within 6 hours of admission and one patient died of streptococcal cellulitis of the neck, after blood had become sterile.

We are highly indebted to Col. J. Norman Walker, C.I.E., I.M.S. (Retd.), Director, Medical and Public Health Department, H. E. H. the Nizam's Dominions for giving us the necessary facilities in the Isolation Hospital, Lingampalli, Hyderabad.

III. Serology of Plague.

The search for some suitable test-tube method which could take the place of the biological method to test the potency of the antiplague serum manufactured at this Institute has been continued during the year. The need for such a method is urgent. The animals undergoing immunisation receive injunctions at 7 to 10 day intervals. The question that always faces the workers is when to stop immunisation. Each biological test with mice takes over 30 days. While the test is going on the animal receives three or more injections. These may and have on occasions proved too many and the serum thereby has become less potent. Obviously if a suitable test tube reaction could be devised it would be of great value. Further at present we do not possess any precise knowledge as to the exact relationship between the phenomenon of active immunity and the so-called serological reactions. The successful standardisation of a biological method offers the possibility of these ralationships to be more clearly studied.

The agglutination reaction and the precipitin reaction have been studied further during the year. The results of the latter are specially encouraging but enough work has not been done to express a precise opinion.

A. Agglutination Reaction.

(WATS.)

A thick suspension of the strain of *Past. pestis* used here for the test is inoculated on acid agar in a Roux flask and incubated at 25°C. for 4 days. The resulting growth is washed off with about 30 c.c. of distilled water containing 1 per cent. phenol. This emulsion is poured into a large test-tube containing glass beads, stored for 6 hours at room temperature (27°-29°C.) and shaken from time to time during the first two hours. The supernatant milky but homogeneous part is decanted from the top without disturbing the bottom layer containing flakes of the growth. An equal quantity of formal saline is added to it to bring down the concentration of the phenol. When required this suspension is shaken and diluted with normal saline containing 0·2 per cent. of formalin, to bring down the opacity to No. 3 of Brown's opacity tube and poured into a large test tube containing glass beads. It is allowed to stand for 48 hours at room temperature, allowing further the coarse particles to settle down. The uniform emulsion thus produced in the upper 3/4ths of the fluid is used for the agglutination test. Its opacity lies between 2 and 3 of the Brown's opacity tube. If the technique is rigidly adhered to, the resulting opacity is constant.

Before adopting the above technique an investigation was undertaken to test the most suitable medium and the temperature for growth. It was found after a series of experiments that the culture on acid agar when incubated at 25°C. was the best. When tested simultaneously, the suspension prepared as above showed some appreciable difference to the one described by Greval and

Dalal (Ind. Jour. Med. Res. XXI, 283, 1933). Parallel tests are being now carried out with the new as well as with Greval's technique. The results so far obtained do not show the reaction to be sensitive enough to serve the purpose in view.

B. *Precipitin Reaction.*

(WAGLE.)

As precipitin reaction has proved to be of value in titration of some of the therapeutic sera, it was thought that a precipitin reaction might be useful for titrating anti-plague serum also. It was necessary first to develop a precipitin reaction, by various methods, then to see if it gives repeatable results and finally to determine which method correlates with the biological test. Extracts prepared by two methods were tried: (1) *Past. pestis*, grown on blood agar at 37°C. for 48 hours, was emulsified in normal saline and incubated at 37°C. for 24 hours. The emulsion was then centrifuged and the supernatent fluid used as antigen (2) another antigen was prepared by emulsifying culture grown on acid agar for 3 days at room temperature (27°C.) in normal saline and heating the emulsion at 100°C. for 1 hour. The heated emulsion was then centrifuged and the supernatent fluid used as antigen.

On testing these antigens with the normal sera and with the immune sera it was found that they give a specific reaction with the antiplague serum. The results given by the same antigen with the same serum were also found to be repeatable. These antigens were therefore put up with antiplague sera obtained from different animals and the titres of the sera noted against the two different antigens. The following table shows the results obtained by the precipitin reaction side by side with those obtained by the biological method :—

Serum from Horse No.	Titre of serum when tested with antigen extracted at 100°C.	Titre of serum tested with antigen extracted at 37°C.	Result of biological potency test.
2	1 in 200	1 in 200 ...	No protection.
7	Less than 1 in 200 ...	Do. ...	Do.
12	1 in 3,200	Do. ...	Good protection.
13	Do.	Do. ...	Do.
15	Less than 1 in 200 ...	Do. ...	No protection.
16	Do. ...	Do. ...	Do.
17	Do. ...	Do. ...	Do.
18	Do. ...	Do. ...	Do.
19	Do. ...	Do. ...	Do.

The results in the table show that the titres of sera obtained with the antigen extracted at 100°C. show close correlation with the biological test done with the same sera. The results therefore so far seem to be encouraging, though the test will have to be applied under different conditions before it will be finally possible to give an opinion as to its practical utility. The work is proceeding on these and other similar lines.

C. Haemolysin Production by Past. pestis. (Wats.)

Bielonowski (1904) considered that the haemolysin content of plague cultures was parallel to their virulence (Arch. Sic. Biol. St. Petersburg. quoted by Diendonne and Otto. 1928).

To test this statement an attempt was made to test the haemolysin content of five truly virulent cultures of *Past. pestis*. tested biologically. The organisms were grown on blood agar, acid agar, acid digest broth and in peptone water for 24 and 48 hours respectively. The red blood cells of sheep, rabbit and rat were used for the test, but the results were repeatedly negative. The filtrates from the 4 and 8 weeks growth in acid digest broth gave similarly negative results.

IV. Epidemiology of Plague.

(SOKHEY AND CHITRE.)

1. *Susceptibility to Plague of Rats from different parts of India.*—In the last report we gave a table showing the relationship between the aggregate human plague death rate per mille in a locality and the percentage of death rate from plague in rats from the same places infected in the laboratory with the standard infective dose. These figures showed that the percentage of deaths from plague among rats after experimental infection had a rough inverse ratio to the aggregate incidence of plague in the area from which the rats were obtained. It will be noticed from the table that Dharamsala had experienced no epidemic of plague during the present pandemic and the percentage of death rate among rats from Dharamsala was shown as 100 per cent.

During the whole of this work, it was the practice to examine batches of about 50 rats from each locality, but in the case of Dharamsala, when we published the table, we had examined a batch of only 17 rats. Therefore we obtained fresh lots of rats from Dharamsala and examined them in the same way. The following table shows the experiments that we carried out with the Dharamsala rats. For each experiment Bombay City rats (as samples of immune rats) and Madras City rats (as samples of highly susceptible rats) were put up as controls. Aggregate plague death rate per mille at the three places is :—Dharamsala 0·00, Madras City 0·396 and Bombay 199·88.

Name of Town.	Number of rats from the locality used for experiment.	Deaths from Plague from experimental infection.	Percentage of deaths.
1st Experiment.			
Dharamsala	17	17	100·0
Madras City	10	7	70·0
Bombay City	10	1	10·0
2nd Experiment.			
Dharamsala	21	17	81·0
Madras City	10	7	70·0
Bombay City	10	2	20·0
3rd Experiment.			
Dharamsala	20	18	90·0
Madras City	10	7	70·0
Bombay City	10	1	10·0
4th Experiment.			
Dharamsala	10	5	50·0
Madras City	10	9	90·0
Bombay City	10	0	0·0
5th Experiment.			
Dharamsala	10	6	60·0
Madras City	10	9	90·0
Bombay City	10	0	0·0
Totals.			
Dharamsala	78	63	80·8
Madras City	50	39	78·0
Bombay City	50	4	8·0

It will be seen that the average percentage death rate for the Dharamsala rats from experimental infection is 80·8 per cent. This experiment shows that even in a place like Dharamsala where no epidemic of plague has been known, there are strains of rats that are naturally immune to plague.

2. *Transmission by heredity of acquired immunity.*—We have carried out experiments to test this possibility. Fortunately we had come across an animal, that is, the laboratory bred white mouse which had during the previous 5 or 6 years shown no natural immunity to plague at all. The batches of mice which we used during this period as controls in our experimental work had almost all died when given the standard infective dose. So we were right in assuming that this animal lacked natural immunity and was suitable for obtaining information on the question of transmission of immunity by heredity. We took 3 separate batches of mice and immunised them with Haffkine Plague Vaccine. Then we gave them our standard infective dose of plague. Those of the animals that survived we bred and the young ones obtained from

these animals were set aside and when they had become 3 to 4 months of age, we infected them with test infective dose and we found that the young did not show any evidence of immunity.

3. *Immunity to Plague of Bombay Rats.*—It had been believed by some previous workers that resistance to plague infection in any given locality was probably due to a mild epizootic going on among the rats at the place. This we believe is not the case. During the last two years we have not come across a single plague infected rat in the City of Bombay out of the 2,000 odd rats collected daily by the Municipality of Bombay and brought to the Institute for examination for plague. We are sure that there is no epizootic going on among the rats of the City of Bombay but they still show a very high degree of immunity to experimental infection.

All the experiments detailed above would seem to indicate how plague epidemics come to an end naturally. The probable explanation seems to be that during an epidemic of plague in any particular locality highly susceptible strains of rats tend to die out leaving behind a higher proportion of naturally resistant strains.

V. Nutrition, Preliminary Experiments.

(MALANDKAR.)

For estimation of Vitamins A and D great stress is always laid on the rearing of a standard stock of white rats on a diet which should allow the animals to reach the standard limits of weight and have free reproduction with sufficient reserves of the vitamins concerned. It has also been noted that a colony stock diet found suitable in one laboratory may not necessarily be suitable in another. Keeping this in view a preliminary study of 4 stock diets has been made, viz., (1) Smith and Chick, (2) Dog Chow, (3) Sherman and Smith and (4) Coward, Cambden and Lee. To meet the local conditions certain modifications were necessary. It was found that the diet No. 4 was the best for the purpose and its consumption value was high as compared to others.

For estimation of Vitamins A and D the technique recommended by the Medical Research Council (Report No. 167) was followed. In the case of Vitamin A the curative method and in the case of Vitamin D the " Line Test " have been studied.

With a view to select a suitable variety of casein for the Vitamin A basal diet, three different brands of casein (1) Merck's pure edible, (2) B. D. H. light white soluble and (3) B. D. H. vitamin and fat free casein were tried. In order to free the casein from possible traces of vitamin A, it was heated in air in thin layers at 110°C. for 8 days.

Six male rats were used in each experiment. The longest depletion period 50 to 58 days was required for rats, parents of which received the Institute diet for rats, which contained traces of Cod Liver Oil. Merck's pure edible casein was used in the basal diet in this case. The weights of rats at the end of the depletion

period were from 106 to 138 g., three rats out of six growing beyond 120 g., the limit allowed by Coward.

The least depletion period 38 to 45 days was for rats receiving the basal diet containing B. D. H. vitamin and fat free casein and produced from parents receiving the Smith and Chick rat diet as the Colony diet. The weights at the end of the depletion period were 108 to 126 g., one rat out of six growing beyond 120 g. The weight at the end of the depletion period according to the Medical Research Council Report should be about 100 g.

VI. Ascorbic Acid content in the blood of normal Indians.

(GOKHALE.)

The bloods of 42 normal young men were examined during the year for their ascorbic acid content. The subjects were young college students between 20 and 30 years of age. The method used for the estimation of the ascorbic acid content was that of Mirsky, Swadesh and Soskin (Proc. Soc. Exp. Biol. Med., 1935, *32*, 1130). The results ranged between 1·20 to 2·48 mg. per 100 c.c. giving an average ascorbic acid content of 1·79 mg. per 100 c.c. of blood.

With the addition of the 38 subjects done last year to these forty-two, eighty normal subjects have been examined so far. The average ascorbic acid content of the blood of the eighty normal young men is 1·80 mg. per 100 c.c. of blood, the figures ranging between 1·20 to 2·54 mg.

VII. Ascorbic Acid content in the urine of normal Indians.

(GOKHALE.)

For this estimation a twenty-four hour collection of urine was used. The urine was collected in a dark coloured bottle using glacial acetic acid as a preservative. The titration technique using the 2 : 6 dichlorophenol indophenol solution was followed (Harris and Ray, Lancet, 1935, I, 71 ; Abbassy, Harris and Ray, Lancet, 1935, II, 1309). The few cases done in the period under report gave for their total ascorbic acid content figures ranging from 5·30 to 12·37 mg.

VIII. Extent of shrinkage of the red cells in blood due to the use of potassium oxalate.

(GOKHALE.)

Determinations were done on ten different samples of blood. Oxalated blood (2 mg. of neutral potassium oxalate per c.c. of blood) was centrifuged against samples of the same blood to which isotonic anticoagulants, heparin or hirudin, had been added. A speed of 4,500 r.p.m. for 30 minutes was used for the determinations.

The results of our determinations of shrinkage in the ten different samples of blood using both heparinized and hirudinized controls are given in the following table :—

Effect of potassium oxalate on cell volume.

No.	Oxalated.	Heparinized.		Hirudinized.	
	Cell volume c.c. per cent.	Cell volume c.c. per cent.	Shrinkage per cent.	Cell volume c.c. per cent.	Shrinkage per cent.
1	38·35	40·75	5·89	40·61	5·57
2	39·71	41·99	5·44	41·95	5·34
3	40·35	42·61	5·30	42·71	5·53
4	45·77	48·58	5·78	48·69	5·99
5	48·99	51·81	5·44	51·89	5·59
6	40·91	43·47	5·89	43·42	5·78
7	40·43	43·06	6·11	42·87	5·70
8	40·57	43·17	6·02	43·02	5·69
9	46·48	49·33	5·79	49·29	5·71
10	41·15	43·95	6·37	43·88	6·22
Average.	42·27	44·87	5·79	44·83	5·71

The values obtained show the shrinkage to be 5·79 per cent. and 5·71 per cent. of the volume of packed cells in heparinized blood and hirudinized blood respectively. The shrinkage is thus practically the same in both cases, the slight difference being within experimental error.

IX. Investigations on the Bio-chemistry of Plant Glycolysis.

(SADASHIVA RAO.)

Practically no direct information is available in this fundamental field of bio-chemistry in spite of the fact that during the last 25 years, and particularly since 1933, enormous and concerted advances have taken place in the closely related fields of alcoholic fermentation and carbohydrate break down in the muscle (Harden, Embden, Meyerhof) and in blood and tumour glycolysis (Warburg). Previously the worker had succeeded in devising a method for obtaining cell-free glycolytic enzyme from germinating peas, and had shown that these enzymes actively break down glucose, fructose and sucrose *in vitro* accompanied by a cycle of esterification and liberation of inorganic phosphate. (Nature, *135*, 909, 1935). The work done since then has followed the problems thus opened up

along the following main lines: (1) The method of isolating the enzymes has been perfected to a point where highly active solutions are obtained, and further purification has been achieved by precipitating with acetate buffer or acetone as well as by absorption on alumina gels, etc. (2) The end-product of anærobic glycolysis has been found to be lactic acid, and not carbon dioxide as is believed to be, though the latter is the observable product in the intact seed, and probably is produced under certain unknown conditions in the extract. (3) Plant metabolism being primarily a carbohydrate metabolism, the cell-free extract has permitted the direct study of the interesting question what sugars and polysaccharides can serve as glycolytic (and presumably, respiratory) substrates. (4) The extracts have made it possible to attempt the all-important question: Is phosphyorylation a *sine qua non* of carbohydrate degradation in the higher plant? A phosphorylating mechanism has been investigated and several phosphoric esters have been isolated and studied. The application of the Embden-Meyerhof scheme to the process is being worked out. (5) The possibility of the occurrence of the Pasteur-Meyerhof reaction in these extracts has been more fully tried. The results of the different aspects of the work will be published when completed.

X. Carbohydrate reactions of Meningococcus.

(WAGLE.)

Meningococcus has attracted attention in India since the epidemic of cerebro-spinal meningitis three years ago. Since that time occasions for the investigation of a meningococcus carrier have arisen here twice. During these investigations it had been observed that in the investigations of such carriers, carbohydrate reactions are a necessity. Difficulty however often arose as to the most suitable medium for carrying out these reactions. Different workers have used different media for this purpose and one is at a loss to know which one to choose. For instance Fildes used for his meningococcal work liquid medium containing 1 per cent. serum, and Gordon, one containing 10 per cent. serum. There is another liquid medium which is often recommended and that is the Hiss's serum-water medium. This contains 25 per cent. serum. Other workers prefer solid agar media containing the proper sugar and certain amount of serum. We had a large number of meningococcal strains isolated from the spinal fluid of meningitis cases from different parts of India and it was thought that this was the best opportunity for settling the question of the most suitable medium for the carbohydrate reaction of meningococci. All the strains we had with us were from the actual meningitis cases and were serologically proved to be so. These strains were therefore inoculated into the four different kinds of media abovementioned and their reactions noted from day to day. Three sugars were used for these reactions, e.g., glucose, maltose and sucrose. The cultures were incubated, for a week before being discarded. The cultures

were examined after 2 days, 4 days and 7 days. Among the 43 meningococcal strains so tested, 40 strains gave typical meningococcal reactions in Fildes serum peptone water, while in Gordons serum peptone water only 33 were typical. Next in order came the Hiss's serum water wherein 23 strains showed appropriate reactions, while in the case of solid agar medium containing serum and sugar, only 16 gave the meningococcal reactions. The media of preference therefore are the peptone waters containing 1 to 10 per cent. serum. The Hiss's serum water and the solid sugar media are not so useful. It is essential in all these cases, however, to incubate the cultures for at least 4 days before discarding them as negative. Another point that is of importance is to make sure of profuse growth in the medium before discarding a reaction as negative.

XI. Pharmacology of Plasmoquine.

(DIKSHIT.)

Investigations on the general pharmacological action of plasmoquine have been completed and the results published in the Records of the Malaria Survey of India. The toxicity of the drug was studied in cats, dogs, guinea-pigs and rabbits. Toxicity tests on protozoa and bacteria showed that plasmoquine is not very toxic to these. Plasmoquine evidently does not act on the malarial parasite as a protoplasmic poison. It has no anti-pyretic action of its own but a combination of quinine and plasmoquine produces a quicker anti-pyretic effect than quinine alone. Plasmoquine lowers the blood pressure when injected intravenously by a direct action on the heart, as well as by depression of the vasomotor centre. It depresses respiration, slightly constricts the bronchioles and inhibits the movements of the gastro-intestinal tract. The important effect of the drug when given in toxic doses, however, seems to be on the liver which shows a fatty degeneration.

A detailed study of the action of plasmoquine in pregnancy has also been undertaken and a preliminary note regarding the action of plasmoquine in pregnancy has been sent for publication. Successful attempts were made to show the action of plasmoquine on the human uterus and it was found that the human uterus responded to the drug in the same way as the animal uteri. It was further observed that a combination of quinine and plasmoquine mutually antagonises the stimulant effect of each on the isolated uterus, an observation which has a great clinical significance. It was also noted that toxic doses of plasmoquine failed to produce abortion in guinea-pigs, even in cases where the pregnancy was well advanced.

Experiments referred to above deal with the action of plasmoquine in toxic doses. Experiments are in progress to show the effect of plasmoquine in therapeutic doses.

XII. Studies in Acetylcholine.

(DIKSHIT.)

A. *Action of Acetylcholine on the Brain.*

Experiments have been continued to investigate the action of acetylcholine on the brain. Attempts to demonstrate liberation of acetylcholine into the brain by stimulation of the afferent nerves have so far not been successful. Presence of acetylcholine in the cerebro-spinal fluid of dogs can easily be demonstrated by injecting eserine in the experimental animals. In some experiments, results were obtained which suggested that an increased quantity of acetylcholine was present after central vagal stimulation, but further work proved that such an increase may occur even without such stimulation. In these experiments the cerebro-spinal fluid was obtained from the cisterna magna. These observations were found to be in general agreement with workers at the National Institute of Medical Research, Hampstead, which were reported recently.

Experiments were also made to investigate this problem by perfusing the head of dogs with oxygenated warm Locke's solution containing a small quantity of eserine. The results obtained could not justify the conclusion, that central stimulation of afferent nerves liberates acetylcholine into the central nervous system.

Further experiments with a new technique are in progress to see if acetylcholine is liberated into the central nervous system as a result of stimulation of the central ends of afferent nerves.

B. *Relation between acetylcholine content of the brain and the choline esterase of the serum.*

In a paper published previously (Dikshit, J. Physiol., 1934, 80, 409) it was shown that acetylcholine is normally present in the brain. The concentration of acetylcholine in the brain of different species of animals has now been determined and the species arranged according to their acetylcholine content. This order varies in the same way as that found by Stedman and his colleagues for the choline-esterase content of the blood serum. The blood contains a specific enzyme which destroys acetylcholine and it has been found that if the acetylcholine content of the brain is high, the enzyme content of the serum also is high. These findings were reported in a paper read before the Indian Science Congress at Hyderabad (Deccan).

C. *Choline-esterase.*

In collaboration with Mr. H. S. Mahal a study of the enzyme which possesses a specific property of destroying acetylcholine has been started. This enzyme which is named as "choline-esterase" is present in different quantities in different species and also in different individuals of the same species but was found to be fairly constant for the same individual. This confirms the work of

Stedman. The methods used were pharmacological as well as bio-chemical. The pharmacological method consisted in recording the response of an isolated rectus abdominis muscle of the frog (R. tigrina) to a known quantity of acetylcholine and comparing it with the response produced by the same quantity of acetylcholine which was brought in contact with a definite volume of blood for a definite period of time. The results obtained by this method compared very favourably with those obtained by the gaso-metric method used originally by Stedman and modified slightly by Mahal. The latter changed the concentrations of the substrate and the enzyme and thus brought about a good correlation between the two methods, a correlation which was not obtained by other workers. By this double control of experimental procedure we have been trying to determine the physiological and pathological aspects of the choline-esterase. We have found that the enzyme concentration in the blood remains very constant for the same individual. It is not appreciably affected by starvation, changes in temperature, diet or by exercise, etc. The R.B.Cs. contain a higher quantity of enzyme than the plasma and this is true for all species. The activity of the enzyme is not affected by keeping it in the refrigerator for 40 hours, but is diminished if kept at room temperature even for 24 hours. With this preliminary physiological knowledge of the enzyme activity we proceed to see if it was affected in any way by diseased conditions. Some experiments have already been conducted which show that acute toxæmias modify the enzyme concentrations of the blood but these experiments are still in very early stages.

XIII. Pharmacological action of Flavones with special reference to their Anthelminthic Effect.

(MAHAL.)

The study of pharmacology, particularly the anthelminthic action of certain synthetic flavones was undertaken by Mr. Mahal in view of the observation of Ratnagiriswaran *et al* (Biochem. J., 1934, 1964) who observed that calycopterin, the active constituent of *Calcycopteris floribunda* belonged to the flavone group, was a dihydroxy tetramethoxyflavone and possessed anthelminthic properties.

Mr. Mahal further elucidated the constitution of calycopterin to be 6 : 4′—dihydroxy—3 : 5 : 7 : 8—tetramethoxyflavone. Besides this he synthesised other hydroxy and methoxy flavones and observed that none of these synthetic flavones nor calycopterin itself had any anthelminthic action as stated by the previous authors. His observations have been further confirmed by a worker in Switzerland (private communication) who has also observed that calycopterin does not possess any anthelminthic action. The diuretic action of these flavones is being studied and has indicated positive results. These results will be published shortly.

XIV. Prophylaxis of Chicken-pox by Inoculation with Vesicular Fluid.

(WATS AND DALAL.)

The application of prophylactic measures for chicken-pox are considered unnecessary owing to the general mildness of its attacks. However, in certain cases, such as in a regiment for example, prophylactic measures are very desirable. There are a number of instances in literature of vaccination with vesicular fluid, but the experiments are not conclusive due to lack of control experiments. A mild outbreak of chicken-pox on board I.M.M.T.S. "Dufferin" gave an opportunity of trying the intradermal methods to protect the contacts. Two series of experiments were done. I. Vesicular fluid collected from 2 cases on the 4th day of the disease was diluted with normal saline to 20 per cent. and filtered through a Chamberland L3 candle. This procedure has not been carried out by the previous workers and is a divergence from the normal routine. Sixty-four persons were inoculated with 0·2 c.c. of this fluid, while 6 with only normal saline (controls). About 8 per cent. of the former had headaches in addition to erythema, the other were free from these symptoms. Neither the controls nor the vaccinated contracted chicken-pox during 9 months of observation. II. The serum from chicken-pox cases was diluted with normal saline to 10 per cent., filtered through Chamberland L3 candle, and injected as in the above experiment into 65 contacts. 58 controls were injected with normal saline. There were no headaches. None contracted chicken-pox during 9 months' observation. As no cases of chicken-pox developed in the controls, it is not possible to state if the inoculation had any influence on the epidemic.

XV. Investigations into the Mosquitocidal Value of Indigenous Drugs.

(WATS AND JASWANT SINGH.)

The primary object of this investigation into the mosquitocidal value of indigenous drugs, was to explore the possibility of evolving some efficient formulæ, which an Indian villager may prepare in his own house from easily accessible and cheap ingredients. The use of an efficient insecticide as an anti-malarial measure is of considerable value in the case of anophelene and other potentially infective mosquito species that may remain in dwellings some hours after obtaining a blood meal. This measure is meant to supplement antilarval measures, and is particularly useful in sparsely populated tracts, such as are found in parts of India, where the cost of application of antilarval measures is prohibitive.

For this investigation sprays were prepared from 65 different sources as follows:

(1) Alcoholic extracts of different plant parts of *Aconitum napellus* Linn., *Adhatoda vasica* Nees., *Atropa belladonna* Linn., *Cannabis sativa* Linn., *Cantharis*, *Cassia augustifolia* Vahl.,

Psychotria ipecacuanha Stokes., *Colchicum autumnale* Linn., *Datura stramonium* Linn., *Eucalyptus globulus Labill.*, *Ferula fœtida* Regel., *Hyoscyamus niger* Linn., *Podophyllum emodi* Wall., *Rhus toxicodendron* Linn., *Strychnos nux-vomica* Linn. (Concentrations 0·001 to 50 per cent.)

(2) Ethereal extracts of plant parts of *Anona squamosa* Linn., *Calotropis gigantea* Ait., *Chrysanthemum indicum* Linn., *Derris elliptica* Benth., *Derris scandens* Benth. (root), *Derris scandens* Benth. (Crys. subst.), *Derris uliginosa* Benth., *Euphorbia neriifolia* Linn., *Garcinia morella* Desr., *Herpestis monnieria* H. B. and K., *Millettia pachycarpa*, *Mudelea suberosa* Benth., *Pterospermum acerifolium* Willd. (Leaves), *Pterospermum acerifolium* Willd. (Flowers), *Swertia chirala* Buch-Ham., *Tephrosia hamiltonii* J. R. Drumm., *Tephrosia purpurea* Pers., *Tephrosia villosa* Pers., *Vitis setosa* Wall. (Concentrations 5 to 50 per cent.)

(3) Chloroform extracts of *Adhatoda vasica* Nees., *Anona squamosa* Linn., *Datura fastuosa* Linn. (Capsules), *Datura fastuosa* Linn. (Leaves), *Girardinia palmata* Gaudich, *Juglans regia* Linn. (Green shells), *Juglans regia* Linn. (Bark), *Lantana camara* Linn. (Leaves), *Lantana camara* Linn. (Roots) *Lantana camara* Linn. (Flowers), *Thevetia nereifolia* Juss. (Flowers), *Thevetia nereifolia* Juss. (Leaves), *Uraria picta* Desv. (Leaves), *Uraria picta* Desv. (Stem). (Concentrations 5 to 50 per cent.)

(4) Kerosene oil extracts and mixtures of *Boenninghausenia albiflora* Reichb., *Cinnamomum camphora* T. Nees. and Eberm., *Nicotiana tabacum* Linn., *Semecarpus anacardium* Linn. (Concentrations 1 to 55 per cent.)

(5) Oils, etc., of *Brassica juncea Coss.* (seeds), *Eugenia caryophyllata* Thumb., *Chenopodium ambrosioides* Linn., *Croton tighium* Linn., *Cinnamomum zeylanicum* Nees., *Gaultheria fragrantissima* Wall., *Pinus longifolia* Roxb. (Concentrations 1 to 50 per cent.)

(6) Miscellaneous chemicals, e.g., Acid salicylic, Benzol trichloride, Bromine, Carbon tetrachloride, Chlorine, Iodine, Naphthaline, Quinine sulphate and Thymol.

The method used in assaying the insecticidal value of the spray was that of Sinton and Wats (Rec. Mal. Surv. India, 5, 275, 1935). One c.c. of the undiluted test solution was sprayed with a No. 15 Devilbiss sprayer into a test chamber into which 25 anophelene mosquitoes (A. annularis or A. sub-pictis) had been introduced. The insects were collected after 30 minutes' exposure and placed in a "mosquito hotel" (Sinton) in the dark for 24 hours, the same number of control insects being placed in the same conditions at the same time. The number of insects dead, moribund and flying were noted at the end of the time. Separate blanks were done with alcohol, ether, kerosene and other extractives.

The results in general showed that all the tested sprays were much inferior to a standard mosquitocide consisting of 1 c.c. of a 1 per cent. solution of Pyrocide 20 in kerosene. The Indian

species of Derris were found to be of poor value; even as contact poisons they produced a mortality of only 60 per cent. Some of the drugs which are well-known fish poisons were found to be poor insecticides.

XVI. The Prevention of Hæmoglobinuria in P. Knowlesi Infections by Methylene Blue and its Curative value when combined with Quinine Salts.

(WATS AND HARBHAGWAN.)

The use of methylene blue for the treatment of malaria has been known for a long time. Experiments were carried out to test the use of methylene blue to prevent the formation of methemoglobin or to ward off other toxic effects produced by the administration of plasmoquine. Monkeys, *Silenus rhesus* infected with *Plasmodium knowlesi* and forcibly fed with plasmoquine were used in this investigation. As it was not possible to reproduce the toxic effects noted in human beings, these experiments were inconclusive but it was observed that the administration of methylene blue solutions eliminated the appearance of hæmoglobinuria. Further experiments as below were undertaken to test this observation. The monkeys were inoculated intraperitoneally with 0·5 c.c. of the blood of a monkey known to be infected with *P. knowlesi*. Daily examination of the blood smears at the beginning of the infection, and weekly examinations subsequently were done. The infection usually developed on the 7th day, and on the 3rd day of the disease about 70 per cent. of the corpuscles were infected. At this stage different animals were treated as follows: (1) Quinine given orally, (2) Quinine given orally + 5 c.c. of 1 per cent. methylene blue in normal saline given intravenously, (3) 5 c.c. of 1 per cent. methylene blue given intravenously, alone.

In all cases quinine did not check hæmoglobinuria and even appeared (though more experiments are required to prove this) to expedite its onset; the monkeys mostly died. Methylene blue alone checked hæmoglobinuria effectively but had no effect on plasmodia, the monkeys dying of infection. However when quinine and methylene blue were given together the monkeys showed no hæmoglobinuria and recovered from the infection. The experiments could not be continued further, but the results undoubtedly indicate that the administration of methylene blue simultaneously with quinine not only enhances its anti-malarial value but checks any tendency to produce hæmoglobinuria in the animals.

LIST OF PAPERS PUBLISHED FROM THE HAFFKINE INSTITUTE DURING THE YEAR 1936.

1. *Dikshit, B. B.*—"Pharmacological Action of Plasmoquine". Records of Malaria Survey of India, *6*, 3, p. 453.

2. *Dikshit, B. B.*—"A Preliminary Note on the Relation between the Acetylcholine Content of the Brain and the Choline

Esterase Concentration of the Serum ". Sent to the Indian Science Congress for January 1937 session.

3. *Sokhey, S. S.*—" Un nouveau serum antipesteux " Bull. mens. de l'Off. Internat. d'Hyg. pub., XXVIII, 1078, 1936.

4. *Sokhey, S. S. and Maurice, H.*—Sur les pouvoirs protecteurs relatifs de vaccins antipesteux preparés les uns au moyen de Cultures tuées par la Chaleur les autres au Moyen de Cultures vivantes avirulentes. (On the relative Protective Powers of Plague Vaccines made of Heat-killed Cultures and those made of Live Avirulent Cultures.) Sent for publication to Bull. mens. de l'Off. Internat. d'Hyg. pub.

5. *Soparkar, M. B.*—" The Filterable forms of the Tubercle Bacillus ". Second International Congress for Microbiology, London, 1936. Rep. of Proc., p. 59.

6. *Soparkar, M. B.*—" The Nature of allergic Reaction in Tuberculosis ". Proc. Ind. Sci. Congr., 1936, p. 486.

7. *Soparkar, M. B.*—" Two Easy Methods of Transplanting Bacilli directly from solid to liquid Culture Media ". Ind. Journ. Med. Res. *24*, 3, 1936.

8. *Wats, R. C. and Bilderbeck, C. W. L.*—" Some Experiments with Entoray machine as an antimosquito measure ". Records of Malaria Survey of India, Vol. 6, 4th December, 1936.

9. *Wats, R. C.*—" An Investigation into the mosquitocidal Value of indigenous Derris and other Drugs ". Records of Malaria Survey of India. (In Press.)

10. *Wats, R. C. and Dalal, N. P.*—" Prophylaxis of Chicken-pox by inoculation with vesicular fluid ". Indian Medical Gazette. (In Press.)

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PART III.

APPENDIX.

Statistics of Anti-rabic Inoculation.

(Standard Tables approved for use in India.)

1935.

APPENDICES.

TABLE I.

A General Statement regarding Patients treated at the Institute and its Centres from 1922 to 1935.

Year.		Europeans.				Asiatics.				Europeans and Asiatics combined.			
		Total treated.	Total deaths.	Total death rate per cent.	Advice cases.	Total treated.	Total deaths.	Total death rate per cent.	Advice cases.	Total treated.	Total deaths.	Total death rate per cent.	Advice cases.
1922...	Institute ...	64	.....	.....	.....	365	4	1·04	175	429	4	0·89	175
	Centres ...	7	.....	.....	.....	236	.....	.....	.....	243	.....	.....	.....
	Combined ...	71	.....	.....	.....	601	4	0·66	175	672	4	0·61	175
1923...	Institute ...	115	.....	.....	59	730	10	1·37	359	845	10	1·18	418
	Centres ...	38	.....	.....	.....	1,521	12	0·78	260	1,559	12	0·76	260
	Combined ...	153	.....	.....	59	2,251	22	0·97	619	2,404	22	0·91	678
1924...	Institute ...	62	1	1·61	41	525	9	1·71	372	587	10	1·70	413
	Centres ...	85	.....	.....	.....	2,880	28	0·97	330	2,965	28	0·94	330
	Combined ...	147	1	.....	41	3,405	37	1·08	702	3,552	38	1·06	743
1925...	Institute ...	42	.....	.....	27	583	6	1·02	219	625	6	0·96	246
	Centres ...	187	.....	.....	5	3,569	28	0·78	393	3,756	28	0·78	398
	Combined ...	229	.....	.....	32	4,152	34	0·81	612	4,381	34	0·79	644
1926...	Institute ...	60	.....	.....	51	588	5	0·87	440	648	5	0·76	491
	Centres ...	287	.....	.....	20	3,861	25	0·64	322	4,148	25	0·60	342
	Combined ...	347	.....	.....	71	4,449	30	0·67	762	4,796	30	0·60	833
1927...	Institute ...	51	.....	.....	37	678	6	0·88	468	729	6	0·82	505
	Centres ...	303	.....	.....	31	3,857	25	0·64	490	4,160	25	0·62	521
	Combined ...	354	.....	.....	68	4,535	31	0·68	958	4,889	31	0·61	1,026
1928...	Institute ...	39	.....	.....	40	679	6	0·88	418	718	6	0·83	458
	Centres ...	216	.....	.....	27	4,166	34	0·81	467	4,382	34	0·77	494
	Combined ...	255	.....	.....	67	4,845	40	0·82	885	5,100	40	0·78	952
1929...	Institute ...	35	.....	.....	43	650	5	0·77	522	685	5	0·73	565
	Centres	286	.....	.....	19	4,621	43	0·93	507	4,907	43	0·89	526
	Combined ...	321	.....	.....	62	5,271	48	0·91	1,029	5,592	48	0·89	1,091
1930...	Institute ...	26	.....	.....	34	710	.....	.....	401	736	.....	.....	435
	Centres ...	314	1	0·29	10	4,692	50	1·07	92	5,006	51	1·01	102
	Combined ...	340	1	0·29	44	5,402	50	0·98	493	5,742	51	0·89	537

1931...	Institute ...	41	.....	.....	42	1,020	3	0·29	394	1,051	3	0·28	436
	Centres ...	328	.....	.....	3	5,670	40	0·70	80	5,998	40	0·68	83
	Combined ...	369	.....	.....	45	6,690	43	0·64	474	7,059	43	0·60	519
1932...	Institute ...	40	.....	.....	52	1,207	3	0·24	457	1,247	3	0·24	509
	Centres ...	252	.....	.....	8	5,984	60	1·00	35	6,236	60	0·96	43
	Combined ...	292	.....	.....	60	7,191	63	0·87	492	7,483	63	0·84	552
1933...	Institute ...	50	.....	.....	62	1,535	2	0·13	722	1,594	2	0·12	784
	Centres ...	288	.....	.....	8	6,139	31	0·50	32	6,437	31	0·48	40
	Combined ...	357	.....	.....	70	7,674	33	0·43	754	8,031	33	0·41	824
1934...	Institute ...	45	.....	.....	49	1,785	4	0·22	665	1,830	4	0·21	714
	Centres ...	344	.....	.....	24	5,462	23	0·42	21	5,806	23	0·39	45
	Combined ...	389	.....	.....	73	7,247	27	0·37	686	7,636	27	0·35	759
1935...	Institute ...	70	.....	.....	64	2,202	4	0·18	593	2,272	4	0·17	657
	Centres ...	306	.....	.....	7	5,544	32	0·57	44	5,850	32	0·54	51
	Combined ...	376	.....	.....	71	7,746	36	0·46	637	8,122	36	0·44	708
Grand Total ...		4,000	2	.....	763	71,459	498	0·69	9,278	75,459	500	0·66	10,041

TABLE II.

A general classification of patients attending the Institute during 1935.

Numerator=Deaths. *Denominators*=Cases.

—	Europeans.					Asiatics.				
	Class of case treated.					Class of case treated.				
	I	II	III	IV	Total.	I	II	III	IV	Total.
Number fully treated	43	9	14	4	70	48	151	1889	4/114	4/2,202
Percentage to total	61·5	12·8	20·0	5.7	...	1·8	6·9	85·9	5·4	...
Total deaths	...	...	...	...	...	...	...	...	4	4
Total death rate per cent.	...	...	...	...	...	...	...	...	3·5	0·18
Percentage of health returns					100					96·7
Number not fully treated (presumably at risk)					1					23
Advice cases (not at risk)					64					593
Cases excluded from the statistical tables for other reasons					...					...
Total ...					65					616
Grand Total ...					135					2,818

TABLE II (*a*).

A general classification of patients attending the Centres during 1935.

Numerators = Deaths. *Denominators* = Cases.

—	Europeans.					Asiatics.				
	Class of case treated.					Class of case treated.				
	I	II	III	IV	Total.	I	II	III	IV	Total.
Number fully treated	212	27	46	21	306	228	2/913	9/3,287	21/1,116	32/5,544
Percentage to total	69·2	8·9	15·1	6·8	...	4·1	16·6	59·2	20·1	...
Total deaths	...	...	...	...	...	...	2	9	21	32
Total death rate per cent.	...	...	...	...	...	...	0·21	0·27	1·8	0·57
Percentage of health returns					93·4					81·0
Number not fully treated (presumably at risk)					27					1,086
Advice cases (not at risk)					7					44
Cases excluded from the statistical tables for other reasons					...					...
Total ...					34					1,130
Grand Total ...					340					6,674

TABLE III.

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators = Deaths. *Denominators* = Cases.

Institute.

A—Animal proved rabid.
B—Animal certified rabid.
C—Animal probably rabid according to history.
D—History of animal unknown.
E—Animal probably not rabid according to history.

—	Europeans.							Asiatics.							Total	Death rate per cent.
	A	B	C	D	E	Total.	Death rate per cent.	A	B	C	D	E	Total.	Death rate per cent.		
Bitten on face	2	...	1	...	...	3	...	2	...	1/18	36	...	1/56	1·7	1/59	1·6
Bitten on trunk and extremities (bare skin).	...	2	10	7	...	19	...	1/43	9	2/183	1,365	...	3/1,660	0·18	3/1,619	0·18
Bitten on trunk and extremities (through clothing).	...	2	2	...	...	4	...	16	...	22	460	...	498	...	502	...
"Licks" on any part of the body.	42	1	1	...	...	44	...	32	10	4	2	...	48	...	92	...
Total ...	44	5	14	7	...	70	...	1/93	19	3/227	1,863	...	4/2,202	...	4/2,272	...
Percentage to total ...	62·9	7·1	20·0	10·0	...	...	...	4·2	0·9	10·1	84·8	...	..	...	...	...
Death rate ...	...	...	...	...	...	...	...	1·0	...	1·3	...	...	0·18	...	0·17	...

TABLE III (*a*).

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators = Deaths. *Denominators* = Cases.

Centres.

A—Animal proved rabid.
B—Animal certified rabid.
C—Animal probably rabid according to history.
D—History of animal unknown.
E—Animal probably not rabid according to history.

—	Europeans.							Asiatics.							Total.	Death rate per cent.
	A	B	C	D	E	Total.	Death rate per cent.	A	B	C	D	E	Total.	Death rate per cent.		
Bitten on face	2	2	4	...	...	8	...	1/16	10	15/256	43	...	16/325	4·9	16/333	4·8
Bitten on trunk and extremities (bare skin).	2	...	40	17	...	59	...	23	12	13/2,880	1/531	...	14/3,446	0·40	14/3,505	0·39
Bitten on trunk and extremities (through clothing).	...	...	11	18	...	29	...	8	...	2/815	728	...	2/1,551	0·12	2/1,580	0·10
"Licks" on any part of the body.	55	58	69	20	8	210	...	31	62	107	22	...	222	...	432	...
Total ...	59	60	124	55	8	306	...	1/78	84	30/4,058	1/1,324	...	32/5,544	0·57	32/5,850	0·54
Percentage to total ...	19·2	19·7	40·6	17·9	2·6	...	...	1·3	1·5	73·1	24·1	...	...	...	...	...
Death rate ..	...	...	...	...	...	...	...	1·2	...	0·73	0·07	...	...	...	...	...

TABLE IV.

Age and sex distribution of patients treated at the Institute.

Numerator = Deaths. Institute. *Denominators* = Cases.

Age in years.	Europeans.				Asiatics.				Europeans and Asiatics combined.	
	Males.	Females.	Total.	Death rate per cent.	Males.	Females.	Total.	Death rate per cent.	Total.	Death rate per cent.
0—4 ...	3	2	5	.....	37	28	65	.....	70	.....
5—9 ...	5	1	6	.....	2/249	84	2/333	0·60	2/339	0·58
10—14 ...	2	3	5	.....	230	61	291	.....	296	.....
15—19 ...	2	2	4	.....	163	35	198	.....	202	.....
20—29 ...	10	11	21	.....	453	72	525	.....	546	.....
30—39 ...	5	5	10	.....	2/314	73	2/387	0·51	2/397	0·50
40—49 ...	5	8	13	.....	177	64	241	.....	254	.....
50—59 ...	1	1	2	.....	64	41	105	.....	107	.....
60—69 ...	3	1	4	.....	21	25	46	.....	50	.....
70 and over ...	.....	.....	.....	.....	9	2	11	.....	11	.....
Total ...	36	34	70	.....	4/1,717	485	4/2,202	0·18	4/2,272	0·17

TABLE IV-a.

Age and sex distribution of patients treated at the Centres.

Numerator = Deaths. *Denominators* = Cases.

Centres.

Age in years.	Europeans.				Asiatics.				Europeans and Asiatics combined.	
	Males.	Females.	Total.	Death rate per cent.	Males.	Females.	Total.	Death rate per cent.	Total.	Death rate per cent.
0—4 ...	12	5	17	.....	175	1/69	1/244	0·40	1/261	0·38
5—9 ...	13	24	37	.....	3/735	2/187	5/922	0·54	5/959	0·52
10—14 ...	11	7	18	.....	1/671	126	1/797	0·12	1/815	0·12
15—19 ...	4	11	15	.....	3/335	79	3/414	0·72	3/429	0·69
20—29 ...	63	25	88	.....	3/1007	5/200	8/1207	0·66	8/1295	0·61
30—39	53	33	86	.....	3/812	162	3/974	0·30	3/1060	0·28
40—49 ...	23	9	32	.....	2/446	2/124	4/570	0·70	4/602	0·66
50—59 ...	8	3	11	.....	4/202	2/77	6/279	2·1	6/290	2·0
60—69 ...	1	1	2	.....	62	1/27	1/89	1·1	1/91	1·0
70 and over ...	.....	.....	.....	.....	30	18	48	.....	48	.....
Total ...	188	118	306	.....	19/4475	13/1,069	32/5544	0·57	32/5850	0·54

TABLE V.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Europeans.
Institute.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg	...	3	6	1	1	...	2	...	...	1	...	...	...	...	...	...	14	...
Trunk	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
Arm	44	3	2	2	...	...	...	...	...	...	...	1	...	...	...	...	52	...
Multiple ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Face	...	1	1	...	...	...	1	...	...	...	...	...	...	...	...	...	3	...
Multiple face ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	44	7	10	3	1	...	3	...	...	1	...	1	...	...	...	...	70	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Separate table for Europeans and Asiatics ; also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) „ —Centres.
(V-b) Asiatics —Institute.
(V-c) „ —Centres.

TABLE V-a.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Europeans.
Centres.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg	...	17	16	5	2	...	...	...	...	...	...	...	1	...	...	...	41	...
Trunk	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Arm	210	22	13	3	1	1	...	1	1	1	...	...	1	...	...	...	254	...
Multiple ...	...	...	2	...	...	...	1	...	...	...	...	...	...	...	...	...	3	...
Face	...	3	2	2	...	...	...	...	...	...	...	...	...	...	...	...	7	...
Multiple face ...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	1	...
Total ...	210	42	33	10	4	1	1	1	1	1	...	...	2	...	...	...	306	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Separate table for Europeans and Asiatics ; Also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) ,, —Centres.
(V-b) Asiatics —Institute.
(V-c) ,, —Centres.

TABLE V-b.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Institute.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg ...	...	246	578	297	260	101	52	30	4	1/4	1	3	1	...	...	1	1/1578	0·60
Trunk ...	...	9	16	6	10	...	1	1	2	...	...	...	...	...	...	...	45	.....
Arm ...	48	95	133	63	71	21	18	10	1/6	2	1	4	...	...	...	1	1/473	0·21
Multiple ...	...	...	4	3	14	7	8	4	2	1	1/4	1	2	...	...	...	1/50	2·0
Face ...	...	9	20	8	7	5	1/1	...	...	...	...	...	...	...	...	...	1/50	2·0
Multiple face ...	...	...	1	...	2	1	1	...	...	1	...	...	...	...	...	...	6	.....
Total ...	48	359	752	377	364	135	1/81	45	1/14	1/8	1/6	8	3	...	...	2	4/2202	0·18
Percentage death rate.	...	...	...	...	...	...	1·2	...	7·1	12·5	16·6	...	...	...	...	...	...	.....

Separate table for Europeans and Asiatics: Also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) „ —Centres.
(V-b) Asiatics—Institute.
(V-c) „ —Centres.

TABLE V-c.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Centres.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg ...	...	1/505	2/1114	1/757	3/571	2/158	101	45	36	7	14	4	8	3	...	3	9/3326	0·27
Trunk ...	...	37	66	49	31	11	6	...	3	1	1	...	...	...	...	...	205	...
Arm ...	222	197	1/379	1/215	1/205	81	51	25	15	7	5	7	8	1	...	6	3/1424	0·21
Multiple ...	...	...	18	34	1/48	45	1/42	1/23	14	9	1/14	2	8	...	...	7	4/264	1·5
Face ...	...	40	1/58	1/59	5/40	13	2/12	3	1/2	2	...	...	...	...	...	...	10/229	4·3
Multiple face ...	...	...	1/4	1/15	14	1/14	1/22	4	7	1/3	1/4	1	2	...	1	5	6/96	6·2
Total ...	222	1/779	5/1639	4/1129	10/909	3/322	4/234	1/100	1/77	1/29	2/28	14	26	4	1	21	32/5544	0·57
Percentage death rate.	...	0·12	0·30	0·35	1·1	0·93	1·7	1·0	1·3	3·4	5·2	...	...	...	...	...	...	...

Separate table for Europeans and Asiatics: Also for Institute and Centres.

(V) Europeans—Institute.
(Va) ,, —Centres.
(Vb) Asiatics—Institute.
(Vc) ,, —Centres.

TABLE VI.

Classification according to number and depth of wounds and interposition of clothing

Numerators = Deaths. Europeans. *Denominators* = Cas

Institute.

Deep Bare.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate
+ +	...	4	3	1	...	...	2	...	...	1	...	...	1	...	...	...	12	*Nil.*
+ −	...	...	1	1	...	...	1	...	...	...	...	...	...	...	...	...	3	"
− +	44	3	3	1	...	...	...	...	...	...	...	...	...	...	...	...	51	"
− −	...	...	3	...	1	...	...	...	...	...	...	...	...	...	...	...	4	"
Total ...	44	7	10	3	1	...	3	...	...	1	...	...	1	...	...	...	70	"
Percentage death rate	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	"

TABLE VI-a.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Deep Bare.	Number of wounds.																Total.	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	...	4	8	4	1	1	1	1	...	...	...	...	1	...	...	...	21	...
+ —	...	4	7	1	...	...	...	...	...	...	...	...	...	...	...	...	12	...
— +	210	24	13	3	3	...	...	...	1	1	...	...	1	...	...	...	256	...
— —	...	10	5	2	...	...	...	...	...	...	...	...	...	...	...	...	17	...
Total ...	210	42	33	10	4	1	1	1	1	1	...	...	2	...	...	...	306	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VI-b.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Institute.

Deep Bare.	Number of wounds.																Total	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	...	235	514	276	272	108	1/59	35	1/10	1/8	5	6	3	...	...	2	3/1533	0·13
+ —	...	59	118	82	81	25	21	10	3	...	1/1	2	...	...	...	...	1/402	0·24
— +	48	36	73	8	4	1	...	...	...	...	...	...	...	...	...	...	170	...
— —	...	29	47	11	7	1	1	...	1	...	...	...	...	...	...	...	97	...
Total ...	48	359	752	377	364	135	1/81	45	1/14	1/8	1/6	8	3	...	...	2	4/2202	0·18
Percentage death rate.	...	...	...	...	...	...	1·2	...	7·1	12·5	16·6	...	...	...	...	...	...	...

TABLE VI-c.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Centres.

Deep Bare.	Number of wounds.																Total	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	...	344	5/857	3/677	10/508	3/193	3/150	65	1/41	1/23	2/33	8	19	3	...	15	28/3021	0·92
+ —	...	157	295	179	161	74	39	25	20	3	3	3	5	1	1	3	969	...
— +	222	158	274	125	78	29	1/26	1/6	8	2	1	1	1	...	...	2	2/533	0·21
— —	...	1/120	213	1/148	77	26	19	4	8	1	1	2	1	...	...	1	2/621	0·32
Total...	222	1/779	5/1639	4/1129	10/909	3/322	4/234	1/100	1/77	1/29	2/38	14	26	4	1	21	32/5544	0·57
Percentage death rate.	...	0·12	0·30	0·35	1·1	0·93	1·7	1·0	1·3	3·4	5·2	...	...	...	...	...	...	...

TABLE VII.

Classification according to number of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Institute.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ...	2	3	3	2	1	...	2	...	...	...	...	...	1	...	...	...	14	...
1 ...	4	1	4	1	...	...	1	...	...	...	...	...	...	...	...	...	11	...
2 ...	7	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	9	...
3 ...	12	2	...	...	...	...	...	...	...	1	...	...	...	...	...	...	15	...
4 ...	4	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	6	...
5 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
6 ...	10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	10	...
7 ...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	...
8 to 14 ...	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3	...
15 to 21 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
22 to 28 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
29 to 35 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
36 to 42 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
50 and over...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	44	7	10	3	1	...	3	...	...	1	...	...	1	...	...	...	70	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VII-a.

Classification according to number of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ...	38	13	5	2	...	...	...	1	1	...	...	...	...	...	...	...	60	...
1 ...	46	10	12	2	3	...	...	...	...	1	...	...	1	...	...	...	75	...
2 ...	34	5	4	1	1	1	1	...	...	...	...	...	...	...	...	...	47	...
3 ...	17	1	2	3	...	...	...	...	...	...	...	...	1	...	...	...	24	...
4 ...	10	2	2	1	...	...	...	...	...	...	...	...	...	...	...	...	15	...
5 ...	14	6	3	...	...	...	...	...	...	..	...	...	...	...	...	...	23	...
6 ...	12	5	1	1	...	...	...	...	...	...	...	...	...	...	...	...	19	...
7 ...	18	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	18	...
8 to 14 ...	18	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	18	...
15 to 21 ...	3	...	4	...	...	...	...	...	...	...	...	...	...	...	...	...	7	...
22 to 28 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
29 to 35 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
36 to 42 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
50 and over ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	210	42	33	10	4	1	1	1	1	1	...	...	2	...	...	...	306	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VII-b.

Classification according to number of wounds and lateness of arrival.

Numerators=Deaths. Asiatics. *Denominators*=Cases.

Institute.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.		
0 ...	3	57	143	87	80	40	1/21	13	3	1/5	1/2	1	1	...	...	2	3/458	0·65
1 ...	3	116	321	166	157	61	38	21	1/7	3	2	6	2	...	...	...	1/903	0·11
2 ...	1	44	80	44	41	12	6	3	...	...	1	...	...	...	...	...	232	...
3 ...	4	22	45	22	15	5	5	1	...	...	...	...	...	...	...	...	119	...
4 ...	20	37	25	8	24	4	4	1	...	...	...	...	...	...	...	...	123	...
5 ...	5	16	27	7	11	4	...	2	1	...	...	1	...	...	...	...	74	...
6 ...	6	11	10	8	8	2	1	...	...	...	...	...	...	...	...	...	46	...
7 ...	3	5	11	2	2	3	1	1	2	...	...	...	...	...	...	...	30	...
8 to 14 ...	2	22	48	17	11	2	4	2	1	...	1	...	...	...	...	...	110	...
15 to 21 ...	1	10	15	6	5	1	1	1	...	...	...	...	...	...	...	...	40	...
22 to 28 ...	...	5	5	3	1	...	...	...	...	...	...	...	...	...	...	...	14	...
29 to 35 ...	...	6	9	3	2	1	...	...	...	...	...	...	...	...	...	...	21	...
36 to 42 ...	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	2	...
43 to 49 ...	...	1	5	...	1	...	...	...	...	...	...	...	...	...	...	...	7	...
50 and over ...	...	6	8	4	5	...	...	...	...	...	...	...	...	...	...	...	23	...
Total ...	48	359	752	377	364	135	1/81	45	1/14	1/8	1/6	8	3	...	...	2	4/2202	0·18
Percentage death rate.	...	...	...	...	...	...	1·2	...	7·1	12·5	16·6	...	...	...	...	...	...	...

TABLE VII-c.

Classification according to number of wounds and lateness of arrival.

Numerators=Deaths. Asiatics. *Denominators*=Cases.

Centres.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.		
0 ...	32	34	71	46	1/39	15	12	7	5	2	3	...	1	...	...	2	1/269	0·37
1 ...	25	147	1/344	219	2/178	1/68	1/62	19	16	3	5	5	4	1	...	4	5/1100	0·45
2 ...	25	116	2/293	1/241	3/172	1/54	1/32	19	16	5	9	2	8	1	...	8	8/1001	0·79
3 ...	23	111	245	1/188	133	46	1/42	21	8	6	5	2	3	...	...	3	2/836	0·23
4 ...	21	100	212	1/133	129	40	1/30	1/11	1/9	1/5	5	4	4	...	1	2	5/706	0·70
5 ..	11	53	1/118	77	1/85	1/33	19	6	4	5	2/4	...	1	1	...	1	5/418	1·1
6 ...	8	1/45	70	39	1/37	14	8	5	4	1	2	...	1	...	...	1	2/235	0·85
7 ...	16	28	47	35	23	10	9	2	5	...	2	...	...	...	...	...	177	...
8 to 14 ...	40	83	143	97	1/76	28	9	7	8	2	1	...	2	...	...	...	1/496	0·20
15 to 21 ...	5	34	1/45	23	21	10	7	1	...	...	1	...	1	...	...	...	1/148	0·67
22 to 28 ...	...	4	12	1/2	...	...	1	2	...	...	1	...	...	...	...	...	1/22	4·5
29 to 35 ...	3	8	17	23	9	2	3	...	1	...	...	1	1	1	...	...	69	...
36 to 42 ...	1	3	3	3	1	...	...	...	1	...	...	...	...	...	...	...	12	...
43 to 49 ...	1	3	5	...	1/2	1	...	...	...	...	...	...	...	...	...	...	1/12	8·3
50 and over ...	11	10	14	3	4	1	...	...	...	...	...	...	...	...	...	...	43	...
Total ...	222	1/779	5/1639	4/1129	10/909	3/322	4/234	1/100	1/77	1/29	2/38	14	26	4	1	21	32/5544	0·57
Percentage death rate.	...	0·12	0·30	0·35	1·1	0·93	1·7	1·0	1·3	3·4	5·2	...	...	...	...	...	...	...

TABLE VIII.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators=Deaths. Europeans. *Denominators*=Cases.

Institute.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+ + ...	5	...	5	...	2	...	12	...
+ — ...	1	1	1	...	...	...	3	...
— + ...	4	...	46	...	1	...	51	...
— — ...	4	...	...	...	...	...	4	...
Total ...	14	1	52	...	3	...	70	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE VIII-a.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators=Deaths. Europeans. *Denominators*=Cases.

Centres.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+ + ...	8	...	11	2	...	...	21	...
+ — ...	10	...	2	...	...	...	12	...
— + ...	15	...	232	1	7	1	256	...
— — ...	8	...	9	...	..	...	17	...
Total ...	41	...	254	3	7	1	306	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE VIII-b.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Institute.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+ + ...	1/1070	9	1/355	45	1/48	6	3/1533	0·13
+ − ..	370	...	29	1/3	...	...	1/402	0·24
− + ...	55	26	85	2	2	...	170	..
− − ...	83	10	4	...	...	...	97	...
Total ...	1/1578	45	1/473	1/50	1/50	6	4/2,202	0·18
Percentage death rate.	0·06	...	0·21	2·0	2·0	...	...	...

TABLE VIII-c.

Classification according to position and depth of wounds, and interpositon of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Centres.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Precentage death rate.
+ + ...	7/1,716	75	3/791	3/181	10/179	5/79	28/3,021	0·92
+ − ...	669	87	151	31	22	9	969	...
− + ...	451	15	405	1/34	23	1/5	2/933	0·21
− − ...	2/490	28	77	18	5	3	2/621	0·32
Total ...	9/3,326	205	3/1,424	4/264	10/229	6/96	32/5,544	0.57
Percentage death rate.	0·27	...	0·21	1·5	4·3	6·2	...	...

TABLE IX.

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Institute.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	6	1	5	...	2	...	14	...
1 ...	5	...	5	...	1	...	11	...
2 ...	...	...	9	...	...	...	9	...
3 ...	1	...	14	...	...	...	15	...
4 ...	2	...	4	...	...	...	6	...
5 ...	...	...	...	...	...	...	...	...
6 ...	...	...	10	...	...	...	10	...
7 ...	...	...	2	...	...	...	2	...
8 to 14 ...	...	...	3	...	...	...	3	...
15 to 21 ...	...	...	...	...	...	...	...	...
22 to 28 ...	...	...	...	...	...	...	...	...
29 to 35 ...	...	...	...	...	...	...	...	...
36 to 42 ...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...
50 and over .	...	...	...	...	...	...	...	...
Total ...	14	1	52	...	3	...	70	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE IX-(*a*).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	10	...	49	...	1	...	60	...
1 ...	14	...	59	1	1	...	75	...
2 ...	6	...	39	1	...	1	47	..
3 ...	3	...	20	...	1	...	24	...
4 ...	4	...	11	...	...	...	15	...
5 ...	1	...	21	...	1	...	23	...
6 ...	2	...	14	...	3	...	19	...
7 ...	..	...	18	...	...	...	18	...
8 to 14 ...	...	...	18	...	...	...	18	...
15 to 21 ...	1	...	5	1	...	...	7	...
22 to 28 ...	...	...	...	...	...	...	...	...
29 to 35 ..	...	...	...	...	...	...	...	...
36 to 42 ...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...
50 and over .	...	...	...	...	...	...	...	...
Total ...	41	...	254	3	7	1	306	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE IX-(*b*).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Institute.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	1/313	10	97	1/16	1/18	4	3/458	0·65
1 ...	666	20	1/180	20	16	1	1/903	0·11
2 ...	184	3	36	2	7	...	232	...
3 ...	84	3	28	2	2	...	119	...
4 ...	77	...	43	1	2	...	123	...
5 ...	48	2	22	2	...	...	74	...
6 ...	26	...	17	1	1	1	46	...
7 ...	22	1	5	1	1	...	30	...
8 to 14 ...	79	1	26	2	2	...	110	...
15 to 21 ...	31	1	7	1	...	...	40	...
22 to 28 ...	12	...	2	...	...	...	14	...
29 to 35 ...	15	1	3	1	1	...	21	...
36 to 42 ...	2	...	...	...	...	...	2	...
43 to 49 ...	5	...	2	...	...	...	7	...
50 and over ...	14	3	5	1	...	...	23	...
Total ...	1/1578	45	1/473	1/50	1/50	6	4/2202	0·18
Percentage death rate.	0·06	...	0·21	2·0	2·0	...	...	...

TABLE IX-(c).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Centres.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	141	4	91	8	1/19	6	1/269	0·37
1 ...	687	49	1/238	58	3/44	1/24	5/1100	0·45
2 ...	2/ 91	36	2/253	56	3/48	1/17	8/1001	0·79
3 ...	508	21	221	1/32	1/42	12	2/836	0·23
4 ...	424	24	175	1/37	2/31	2/15	5/706	0·70
5 ...	2/245	18	107	1/28	11	2/9	5/418	1·1
6 ...	2/134	10	67	8	9	7	2/235	0·85
7 ...	106	7	47	6	8	3	177	...
8 to 14 ...	1/297	22	145	17	13	2	1/496	0·20
15 to 21 ...	1/92	8	37	7	3	1	1/148	0·67
22 to 28 ...	1/13	3	5	1	...	...	1/22	4·5
29 to 35 ...	50	...	14	4	1	...	69	...
36 to 42 ...	10	...	2	...	...	...	12	...
43 to 49 ...	8	...	3	1/1	...	...	1/12	8·3
50 and over ...	20	3	19	1	...	...	43	...
Total ...	9/3326	205	3/1424	4/264	10/229	6/96	32/5544	0·57
Percentage death rate.	0·27	...	0·21	1·5	4·3	6·2	...	...

TABLE X.

Deaths from Hydrophobia amongst persons treated in

Europeans.

Institute.

Serial Number.	Case Number.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.					Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
← *Nil* →																					

TABLE X-a.

Deaths from Hydrophobia amongst persons treated in

Europeans.

Centres.

Serial Number.	Case Number.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.					Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
← *Nil* →																					

TABLE X-b.

Deaths from Hydrophobia amongst persons treated in

Asiatics.

Institute.

Serial Number.	Case Number.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.					Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
1	1316	23	M.	9	Dog ...	C	...	...	...	6	6	+	+	+	+	0	2	25	IVf	5 % 7cc.	14 days
2	1872	40	F.	8	Dog ...	A	9	...	...	...	9	+	+	+	–	0	1	41	IVe	,, 5cc.	,, ,,
3	1253	54	M.	30	Dog ...	C	...	...	8	...	8	+	+	+	+	1	2	57	IVe	,, 7cc.	,, ,,
4	1601	65	M.	35	Dog ...	C	...	4	6	...	10	+	+	–	+	0	3	68	IVe	,, ,,	,, ,,

TABLE X-o.

TABLE

Deaths from Hydrophobia amongst

Asia

Cen

Serial No.	Case No.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and of wo		
					Kind.	Class.	Leg.	Trunk.	Arm.
								Died during	
1	117	1	M.	4	Dog ...	B	...	...	...
2	240	2	F.	30	Jackal ...	C	4	...	9
3	15	2	M.	7	Do. ...	C	8	...	...
							Died within 15 days		
1	17	15	F.	2	Dog ...	C	...	...	...
2	45	15	M.	6	Do. ...	C	...	...	...
	335	15	F.	8	Jackal ...	C	...	...	1
4	45	17	M.	35	Do. ...	C	...	...	3
5	182	17	F.	2	Do. ...	C	...	...	4
6	448	20	M.	20	Do. ...	C	...	...	6
7	159	20	F.	45	Dog ...	C	1	...	6
8	39	20	M.	50	Do. ...	C	3	...	...
9	18	21	F.	40	Jackal ...	C	...	...	...
10	215	21	F.	20	Do. ...	C	...	...	3
11	43	22	M.	45	Dog ...	C	2	...	...
12	135	25	M.	30	Jackal ...	C	...	...	2
13	12	25	M.	17	Wolf ...	C	...	...	...
14	411	26	M.	50	Jackal ...	C	...	...	...
15	467	29	F.	25	Dog ...	C	2	...	...
							Died after 15 days		
1	775	26	M.	55	Jackal ...	C	...	...	...
2	435	28	F.	50	Do. ...	C	...	...	1
3	784	30	F.	22	Do. ...	C	...	...	...
4	42	31	F.	9	Dog ...	C	...	...	...
5	814	35	M.	15	Jackal ...	C	3	1	2
6	154	37	M.	20	Do. ...	C	...	...	3
7	268	39	F.	55	Do. ...	C	5	...	...
8	445	40	M.	16	Do. ...	C	1	...	...
9	112	40	M.	45	Dog ...	C	2	...	...
10	462	42	M.	35	Jackal ...	C	4	...	...

X-e.

persons treated in

tics.

res.

position unds.		Wounds.								Treatment.	
Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.	Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Type.	Duration in days.
treatment.											Days.
2	2	+	—	+	+	20	1	22	IVf	5 %	7 cc. 1
...	13	+	—	+	+	15	1	18	III	,,	5 ,, 2
...	8	+	—	+	+	30	1	33	III	,,	5 ,, 2
after treatment.											
2	2	+	+	+	+	2	1	18	IVf	5 %	7 cc. 14
8	8	+	—	+	+	4	1	20	IVf	,,	7 ,, 14
2	3	+	—	+	+	4	2	21	IVf	,,	7 ,, 14
2	5	+	+	+	+	1	2	20	IVf	,,	10 ,, 14
...	4	+	+	—	+	2	1	20	IVe	,,	7 ,, 14
4	10	+	—	+	+	5	1	26	IVf	,,	10 ,, 14
...	7	—	—	+	—	4	3	27	III	,,	5 ,, 14
...	3	—	—	—	+	26	2	48	II	,,	2 ,, 14
4	4	+	—	+	—	2	3	25	IVf	,,	10 ,, 14
	9	+	—	+	+	4	1	26	IVf	,,	10 ,, 14
2	4	+	—	+	+	45	2	69	III	,,	5 ,, 14
...	2	+	—	+	—	1	2	28	IVe	,,	7 ,, 14
4	4	+	+	+	+	2	2	29	IVf	,,	10 ,, 14
4	4	+	—	+	—	1	1	28	IVf	,,	10 ,, 14
...	2	+	+	+	+	2	1	32	IVe	,,	7 ,, 14
after treatment.											
4	4	+	+	+	+	1	2	29	IVf	5 %	10 cc. 14
1	2	+	—	+	+	5	1	34	IVf	,,	10 ,, 14
6	6	+	—	+	+	1	1	32	IVf	,,	10 ,, 14
3	3	+	—	+	+	3	3	37	IVf	,,	7 ,, 14
...	6	+	—	+	+	3	3	41	IVf	,,	7 ,, 14
...	3	+	—	+	+	2	2	41	III	,,	5 ,, 14
...	5	+	—	+	+	2	1	42	III	,,	5 ,, 14
9	10	+	—	+	+	5	2	47	III	,,	5 ,, 14
...	2	+	—	+	+	15	2	57	III	,,	5 ,, 14
...	4	+	—	+	+	6	2	50	III	,,	5 ,, 14

TABLE

Serial No.	Case No.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and of wo		
					Kind.	Class.	Leg.	Trunk.	Arm.
							Died	*after*	*15 days*
11	8	44	F.	65	Wolf ...	C	...	...	...
12	110	46	M.	25	Dog ...	C	5	...	...
13	122	49	F.	20	Do. ...	C	4	...	...
14	66	51	M.	8	Do. ...	A	...	...	...
15	453	57	M.	50	Jackal ...	C	4	...	...
16	539	120	M.	12	Dog ...	C	1	...	...
17	3	178	F.	8	Jackal ...	C	1	...	4

X-c—*contd.*

position unds.		Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
after treatment—continued.											
6	6	+	+	+	+	4	2	50	IVf	5 %	10cc. Days: 14
...	5	+	—	+	+	5	2	51	III	„	5 „ 14
...	4	+	—	+	+	8	3	60	III	„	5 „ 14
4	4	+	+	+	+	0	1	52	IVf	„	7 „ 14
...	4	+	—	+	+	5	3	65	IVe	„	7 „ 14
...	1	+	—	—	—	6	2	128	II	„	2 „ 14
1	6	—	—	—	—	2	4	184	IVf	„	7 „ 14

TABLE

Classification of the animal which bit,

Numerators = Deaths.

Animal.	Institute.								
	Number treated.			Percentage to total.			Death rate per cent.		
	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.
Dog ...	65	4/2,125	4/2,190	92·9	96·6	96·4	...	0·18	0·16
Jackal ...	...	19	19	...	0·9	0·8	...	...	...
Cat ...	5	23	28	7·1	1·3	1·2	...	...	...
Monkey ...	...	16	16	...	0·8	0·7	...	...	...
Fox ...	...	...	...	...	...	...	...	...	...
Man ...	...	6	6	...	0·2	0·2	...	...	...
Donkey ...	...	...	...	...	...	...	...	...	...
Horse ...	...	6	6	...	0·2	0·2	...	...	...
Buffalo ...	...	...	...	...	...	...	...	...	...
Hyena ...	...	...	...	...	...	...	...	...	...
Wolf ...	...	...	...	...	...	...	...	...	...
Cow ...	...	...	...	...	...	...	...	...	...
Tiger ...	...	...	...	...	...	...	...	...	...
Unknown ...	...	7	7	...	...	...	...	...	...
Total ...	70	4/2,202	4/2,272	...	...	...	...	...	...

XI.

scratched or licked the patient.

Denominators = Cases.

Centres.								
Number treated.			Percentage to total.			Death rate per cent.		
Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.
292	12/4,782	12/5,074	95·4	86·2	86·6	...	0·25	0·23
1	18/677	18/678	0·3	12·2	11·5	...	2·6	2·6
6	7	13	1·8	0·1	0·2	...	...	...
2	10	12	0·6	0·1	0·2	...	...	...
...	6	6	...	0·1	0·1	...	...	...
1	24	25	0·3	0·4	0·4	...	...	...
...	10	10	...	0·1	.....	...	...	...
4	10	14	1·2	0·1	.....	...	...	...
...	3	3	...	.....	.....	...	...	...
...	2	2	...	.....	.....	...	...	...
...	2/10	2/10	...	0·1	0·1	...	...	...
...	2	2	...	.....	.....	...	...	...
...	1	1	...	.....	.....	...	...	...
...	.....	.....	...	.....	.....	...	...	...
306	32/5,544	32/5,850	...	.....	.....	...	...	...

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TABLE XII.

Number of patients treated by months.

	Institute.			Centres.			Institute and Centres combined.		
	Europeans	Asiatics.	Total.	Europeans.	Asiatics.	Total.	Europeans.	Asiatics.	Total.
January ...	2	199	201	52	473	525	54	672	726
February ...	7	203	210	28	442	470	35	645	680
March ...	2	238	240	12	460	472	14	698	712
April ...	12	183	195	29	449	478	41	632	673
May ...	4	206	210	15	511	526	19	717	736
June ...	1	183	184	30	433	463	31	616	647
July ...	12	153	165	32	439	471	44	592	636
August ...	8	175	183	32	457	489	40	632	672
September ...	7	163	170	15	445	460	22	608	630
October ...	15	171	186	20	505	525	35	676	711
November ...	...	144	144	22	448	470	22	592	614
December ...	...	184	184	19	482	501	19	666	685
Total ...	70	2,202	2,272	306	5,544	5,850	376	7,746	8,122

TABLE No. XIII.

Statement showing brains submitted and examined for rabies.

1935.

Serial No.	Animal.	Number of brains examined.	Positive.	Negative.	Unfit for examination.
1	Dogs	205	165	37	3
2	Cats	2	2	...	...
3	Beer	1	...	1	...
4	Donkey	1	1	...	...
5	Bull	1	1	...	...
6	Fox	1	1	...	...
7	Human being	2	...	2	...
	Total ...	213	170	40	3

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